PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The Embryology of Pieris rapae. Organogeny

L. E. S. Eastham

Phil. Trans. R. Soc. Lond. B 1931 **219**, 1-50
doi: 10.1098/rstb.1931.0001

PHILOSOPHICAL TRANSACTIONS.

I. *The Embryology of* Pieris rapæ.—*Organogeny.*

By L. E. S. Eastham, *Department of Zoology, Cambridge.*

(*Communicated by* J. Stanley Gardiner, *F.R.S.*)

(Received January 18,—Read June 26, 1930.)

[Plates 1–9.]

Contents.

VOL. CCXIX.—B 462 B [Published October 2, 1930.

1. *Introduction.*

The following is a further account of the embryology of *Pieris rapæ* dealing with development from the time of germ layer formation, EASTHAM, 1927, to the time that those germ layers have become differentiated into the several systems of organs to be found in the caterpillar. Embryological differentiation, *i.e.*, fate of germ layers, rather than histological differentiation is described. Cytological details have been largely ignored, and since germ cell differentiation is so largely a cytological matter this part of the story is treated superficially, with the intention of dealing with it as a special problem later. The interesting morphological subject of head development has been treated at some length on account of the special interest raised by three important recent accounts dealing with this subject—two anatomical, SNODGRASS, 1928, and DENIS, 1927, and the third embryological, by three collaborators, LEUZINGER, WIESMANN and LEHMANN, 1926.

I have to acknowledge my indebtedness to Prof. J. S. GARDINER, F.R.S., for the facilities afforded me for prosecuting the work in the Zoological Laboratories, Cambridge, and for giving me continued access to his garden, where much valuable material was obtained. No special technique was employed beyond that described in my earlier paper 1927. In later stages of development when the embryo has attained considerable rigidity of form, collodion embedding was not found necessary. In transferring from oil to wax it was found desirable to immerse the eggs first in wax, with a certain proportion of oil, and later transfer them to pure wax. In this way convection currents are minimised. For removal of the eggs from the embedding oven, the use of a hot pipette was damaging, causing the delicate tissues to become separated and as a result impossible to interpret in the matter of spatial relations.

2. *Development of Body Form.*

It was seen, EASTHAM, 1927, that after the completion of the gastrulation process, the embryo had a strong dorsal curvature at both ends, the anterior and posterior ends lying on the dorsal side of the egg and pointing towards one another. When all the somites are formed and the appendages are visible as blunt lobes projecting from their segments, the embryo is so long that its head and tail are almost touching. So close do they lie to one another that in taking transverse sections through the egg, the embryo is cut twice in each section except at the place where the narrow gap exists between upturned head and tail. Figs. 1 and 2 (Plate 1) clearly illustrate this feature. At such a stage the dorsal surface of the embryo does not exist. The gross changes in form which now take place are closely associated with the development of the lateral body wall and with the telescoping of segments throughout the body, so that they occupy less space longitudinally.

The approximation of the segments causes the latter to be more clearly defined, since they now become separated by deep but narrow intersegmental furrows. The net result of this economy of space within the egg is that the mouth and anus are carried forwards and backwards respectively so as to face the anterior and posterior poles of the egg, fig. 4. The anus is found on the terminal abdominal segment which occupies the position between dorsal and ventral parts of the germ band owing to the extreme flexure which that part of the embryo underwent in earlier development. It opens into a proctodæum directed backwards. For this part of the embryo, attainment of larval form consists of an unravelling of the coil in which the abdomen finds itself. It follows from this that as the abdomen straightens out the anus will be carried to a dorsal (fig. 3) and then to a terminal position. The proctodæum accordingly changes its orientation and from its first backward and dorsally directed position attains the forward orientation shown in fig. 4. Just as the straightening out of the embryonic abdomen changes the position of the anus and direction of the proctodæum so the shifting of the head segments brings about a change of position of the mouth and a change of orientation of the stomodæum. The mouth is carried from its first dorsal position, fig. 1, to its final ventral one, fig. 6, through the intermediate condition seen in fig. 4.

Abdominal and cephalic blastokinesis are up to this point similar processes, but whereas in the abdomen the whole of the segments participate in this revolution, in the head the morphological anterior border which at first lies above and opposite the labial segment retains this position, and the shifting of the mouth is permitted by the great growth of a facial region between the stationary anterior border of the protocerebral segment and the downward shifting mouth.

It must not be assumed from this that the ultimate preoral region is entirely protocerebral, for as will be seen later the antennary segment attains a secondary preoral position. After the embryo has reached the stage shown in fig. 4, where the anus is in the posterior position, the last two or three segments become completed above, by upward growth of their lateral walls and fusion of these in the mid-dorsal line. These completed segments now become arched upwards, the terminal segment bent downwards. The embryo thus attains the shape of a mark of interrogation lying on its face (fig. 5).

The terminal abdominal segment now turns forward and the head region straightens out, so that at some short time before hatching the embryo is ventrally flexed in the form of a U with its head and tail again in close approximation, but this time on the ventral side of the egg (fig. 6). A final stage differs from this only in the greater elongation of the embryo, resulting in the last two segments being coiled up either on the left or right side of the thorax.

It will be seen that development of body form has involved a complete reversal of position in the egg, a change from dorsal to ventral flexure. The embryo has, however, maintained its position, in that its longitudinal axis coincides with that of the egg all the time. The embryo may be said to have slowly whipped its tail from a dorsal to a ventral position. The reason for this great change of position is to be sought in the development of the body wall which takes place during this time. Even after gastrulation is completed, the ectodermal part consists almost entirely of the ventral body wall, with lateral edges only sufficiently upturned to enclose the small mesodermal somites lying within them.

At the end of embryonic development the ectoderm has grown upwards at each side and turned in towards the mid-dorsal line where fusion has taken place to complete the dorsal body wall. Now if we consider for a moment the condition of the embryo after gastrulation (fig. 1) in the abdominal region, it will be seen that if the lateral body wall develops simultaneously in all segments, that of the first three segments will develop upwards towards the dorsal side of the egg, that of the fourth to sixth segments will develop in a forward direction, and the lateral body wall of the seventh to tenth abdominal segments will grow towards the ventral side of the egg. Growth in all these segments is converging on a restricted region lying within the dorsal curvature of the flexed embryonic abdomen. Such a restricted area offers a serious obstacle to the necessary body wall differentiation. Since the lateral body wall develops nearly simultaneously in the segments concerned, as each segmental ectoderm grows up to its own morphological dorsal side it will tend to crowd out its neighbours, or be crowded out by them. More space is required and the only way to obtain this is by straightening out the embryo so that each segment has a territory with parallel frontiers into which it may expand.

What has been said for the abdomen applies equally well to the head and thorax, these segments forming an anterior curvature similar to that of the abdomen posteriorly. It is therefore quite clear that the body wall differentiation and the requirements of space concerned with this are sufficient to explain the first part of the blastokinesis process. When the embryo has attained its straightened form (fig. 4) the lateral body wall is formed, the dorsal body wall is still absent. The ectoderm has the form of a deep gutter which is open above to the yolk. Needless to say this ectodermal gutter is largely filled with other organs, such as alimentary canal, muscles and trachea, etc., in process of differentiation, but with these we are not for the moment concerned since their development does not impress itself on the external form of the embryo.

Ectodermal growth over the dorsal side of the embryo takes place in such an order that the last segments to be completed are thoracic. Head and abdominal segments both precede these in order of differentiation. In this case, as with midgut formation, development no longer proceeds regularly from before backwards, but is effected simultaneously in the anterior head and posterior abdominal segments. Such developments proceeding from the two ends towards the thoracic region, result naturally in the latter region being completed last. It is important to notice that this, the thoracic region, is the place where cephalic, abdominal and lateral folds of the amnion ultimately fuse, so severing completely the connection between the embryo and the embryonic membranes. It is also important to note that the last part of the embryo to be cut off from the yolk is that which contains within it the major portion of the midgut. From the time that the latter becomes a continuous band of tissue, holding yolk above it, till the above complete closure is effected, the midgut is the only organ which is in direct contact with the yolk. It would seem, therefore, of paramount importance to the welfare of the growing embryo that this midgut region should be the last to be shut out from that yolk which lies between the amnion and serosa, and which lies away from direct contact with the embryo.

How explain the further curvatures of the embryo, which bring the mouth and anus into close proximation the ventral side of the egg ? The main features of head development consist of increase in size of the protocerebral lobes, which ultimately dominate the whole head region, and of the backward shifting of the mouth, so as to leave antennæ in a preoral, and mandibles and maxillæ in a par-oral position. The backward movement of the mouth bringing it to its ultimate ventral position is brought about by growth and the shifting of parts relative to one another, rather than by flexure. To what extent backward movement of the mouth is responsible for increase in the size of the preoral region on the one hand, or how much the backward movement of the mouth is due to preoral or protocerebral growth on the other, is difficult to say. That the antennæ are found in a final preoral position suggests that both factors are operating at once. Abdominal growth is concerned with segmental differentiation in an embryo which is longer than the egg. Ventral development occurs during dorsal flexure and a reversal of this flexure is necessary before dorsal development is possible. The changes of growth rates between ventral and dorsal sides therefore seem to be largely responsible for the caudal flexure of the embryo.

The segmentation of the abdomen into 11 segments is clearly shown in the figs. 1 and 2. Of these the last is absorbed by the developing proctodæum, so that by the time the straight stage (fig. 4) is reached this segment is no longer visible.

3. *Appendages.*

The appendages develop as paired out-growths of the body wall on all segments of the body. All these are at first post-oral, except the labral lobes (if these be considered as appendages). The antennæ, however, quickly assume a preoral position. The pre-mandibular appendages (fig. 7) are extremely small and transient, the ectoderm of this segment being concerned with tritocerebral nervous development, its mesoderm forming the subœsophageal body. In a fully formed embryo and in the hatched larva (figs. 8 and 6), a pair of rounded lobes are found to exist immediately in front of the prothoracic legs. In the embryo they have the appearance of a pair of limb-buds (fig. 9, Plate 2) and might lead one to a false conclusion that an additional head or thoracic segment was involved. Apart from the fact that no separate neuromere or somites exist for such a supposed segment, any doubt as to its real significance is removed by tracing its origin.

In the state shown in fig. 1 it will be seen that the length of the prothoracic segment is greater than that of other segments. As the first walking leg develops, a prominent protuberance appears on the anterior side of its basal joint, fig. 3. The groove which separates this from the basal joint rapidly deepens (figs. 5 and 11), till finally the excrescence finds itself with a separate attachment to the ventro-lateral sides of the prothoracic body wall in front of the legs. The positions of these lobes in the larva is ventral (fig. 8). They are present in all larval instars and are commonly held in a semi-turgid condition during life. The outer surface is well provided with short simple setæ. I have been able to find no reference to these structures and can offer no suggestion as to their functions.

Serially homologous with the limbs of the thorax, limbs occur on the first 10 segments of the abdomen (fig. 1). These never attain any appreciable size, and except for those on the first abdominal segment, at the time when the full number is present, are in the form of short rounded protuberances. The first pair of abdominal legs are bluntly tapering structures, smaller than but similar in form to the thoracic limbs. The limbs on the segments 3 to 6 and 10 persist as the prolegs of the larva, the others are absorbed (fig. 5).

The limbs of the first abdominal segment have a peculiar history. In their first stages they closely resemble normal ectodermal limb outgrowths. But when the embryo begins to straighten out, so effecting the change in form as exhibited by figs. 3 and 5, the free end of the terminal cells of the limb becomes almost impossible to stain and forms a brush-like end to the appendage. The cells with such extensions projecting into the amniotic cavity, now proceed to invaginate, to form a shallow depression at the distal end of the appendage. This process of invagination continues and the cells subjected to it become large and pyriform and deeply embedded in the body cavity. The organ is now in the form of a deep pit with a round neck bounded by ordinary hypodermal cells, the bottom of the pit being formed by the large pyriform cells mentioned above (fig. 10). Through the neck of this pit there pass out the faintly staining extensions of the pyriform cells, and these with care can be traced as far as the amnion, with which they come in contact.

That the extensions of the cells which appear in the form of a brush are not simple secretions is clearly proved by the examination of sections taken straight across the opening of the pit. Such sections show clearly the cell limits even in the faintly staining regions. This peculiar structure attains its maximum development when the embryo is on the point of hatching, and its development coincides with the revolution of the embryo in the yolk. On hatching no further sign of the organ can be found, except a small group of degenerating cells within the body wall in the first abdominal segment. Though abdominal appendages have been described in lepidopterous embryos, *e.g.*, TOYAMA, 1902, and GRABER, 1891, in *Bombyx*, there appears to be no account of the first abdominal appendages in this order behaving in this manner.

Such organs developing on this segment have, however, been found in most insect orders, and it is highly probable that they are universal in the class. HUSSEY, 1926, gives an account of their development in two species of rhynchota, namely, *Belostoma flumineum* and *Ranatra fusca*, and brings together all the published information on these structures on insects as a whole. His conclusions agree with mine, in that in these two insects the organs develop during the revolution of the embryo, attain their maximum development at the end of this period and become functionless and enclosed by the ectoderm after hatching.

Though the abdominal appendages had been investigated before WHEELER's contribution, 1890, it was this author who gave them the name pleuropodia by which they have since become known. The name is descriptive, indicating their foot-like structure and their common tendency to take up a pleural position on the body wall. From HUSSEY's comprehensive review 1926 we find them to exist most prominently in the generalised orders, Orthoptera, Dermaptera, Isoptera, Coleoptera, and Hemiptera. In the Lepidoptera, till my present account, they were regarded as being ill-developed. TOYAMA, 1902, on *Bombyx*, finding no difference between them and the remaining abdominal appendages. In Hymenoptera they have so far only been described as minute envanescent papillæ—the vestiges of legs homologous to those of the cruciform larvæ of sawflies. NELSON, 1915, did not find any abdominal appendages in *Apis*, WHEELER, 1910, and TANQUARY, 1913, found rudimentary papillæ on the first abdominal segments of embryos of *Formica fusca*, *Myrmica scabrinodis* and *Camponotus herculaneus*. There is no account of their presence in Diptera.

DE SELYS LONGCHAMPS made a careful study of Pleuropodia in the beetle *Tenebrio molitor*, 1904. In this form during the course of development, the pleuropodium becomes closely adherent to the embryonic membrane, the amnion breaks down and occupies the cavity of the appendage and the serosa loses differentiation. The amnion and serosa apart from the region of the pleuropodium become fused together and withdraw to the dorsal side of the embryo. From this he concludes that the organ plays an important part in releasing the embryo. That is the tentative conclusion at which I had arrived with regard to the pleuropodium in *Pieris*. Its maximum development coincides with the rupture of the embryonic membranes, its extensions can certainly be traced across the amniotic cavity to the amnion, and it disappears on larval emergence. All these facts support the conclusion of DE SELYS LONGCHAMPS. The evidence is, however, only circumstantial, and certainly in *Pieris* might equally conform to the opinions expressed by KORSCHELT, 1912, and BLUNCK, 1912, that it is an organ which produces a fluid to enable the embryo the more easily to perform the flexures of body effected during blastokinesis, for it has already been pointed out that the gland is in process of development during blastokinesis. BAILLON, 1920, seems to be the only other worker who suggests a different function. In his large work on Locustids he suggests in some species they may have a respiratory function. Here again there is no experimental evidence in support of this assertion.

Any discussion, however brief, on pleuropodia must contain treatment from the morphological, phylogenetic and physiological points of view. Morphologically they are undoubted limbs—hollow extensions of the body wall into which passes a portion of the mesoderm belonging to that segment. They are appendages homodynamous with those of the thorax and head. Phylogenetically there can be no doubt that *as* *limbs* they are vestigial structures, reminiscent of the time when insects or their ancestors bore functional limbs on all abdominal segments. Physiologically, the answer can only at present be guessed. That they are functional in the embryo there can be no doubt, and any attempt such as that of Wheeler, 1890, to regard them as vestigial odoriferous glands or aphrodisiacs seems futile. Wheeler appears to have failed to distinguish between past function as a limb and present function as a gland. Whether the function is respiratory, excretory or secretory, there seems to be no doubt that it is an active and important one in the embryo. This does not preclude us from still regarding the limb (as such) as vestigial, and only larger than the other abdominal limbs on account of its peculiarity of being converted for some developmental purpose. If the gland is vestigial —a relic of an ancestral repugnatorial gland as is also suggested by Wheeler, 1890— it is surely remarkable that its period of development and activity is so similar in all the insects where it has been adequately investigated.

The thoracic limbs quickly attain an appreciable size and from their ventro-lateral attachments pass upwards and backwards along the side of the embryo. The prolegs which are retained on their segments must be regarded as true appendages. They develop in the same manner as those on the head and thorax, have the same relation to their own somites, and a musculature develops in connection with each comparable to that of the thoracic limbs though of a weaker order.

4. *The Mesoblastic Somites.*

In the account of the early embryonic development of *Pieris* (Eastham, 1927) the stage of somite differentiation was reached in which paired somites were formed occupying the lateral pockets of the ectoderm segmentally. In the median region of the embryo, above the neural groove, lies a sheet of mesoderm, continuous along the whole length of the embryo and forming a connecting link between the somites of the two sides (fig. 12). It was pointed out in that account that external segmentation of the embryo preceded somite formation; figs. 13 and 14 clearly demonstrate this point, both being sagittal sections through the embryo. There it is seen that though the ectoderm is strongly segmented and limb buds have begun to grow out, the mesoderm is no more heaped up segmentally within the hollows of the outgrowing limb-buds in limb-bearing segments than elsewhere. In lateral segmental masses of mesoderm at such a stage there is as yet no radial arrangement of cells nor central somitic cavity. A radial or rosette arrangement soon develops in each of these masses, and in the centre of each will be seen a minute space, the cœlomic cavity (fig. 12). At this stage the limbs are well formed and the embryo is in the stage indicated by figs. 1 and 2. If such a stage is examined by means of sagittal sections we shall find that the somites are not arranged in longitudinal series like a series of thick-walled hollow spheres, as might be gathered from purely transverse section examination. In the median line through a longitudinal series of somites the mesoderm is seen to consist of two layers of cells, closely united with one another intersegmentally but becoming separated from one another segmentally.

The cavity formed by this separation is not spherical but extends far into the intersegmental regions (fig. 15) and only just fails to be continuous throughout, from one segment to another.

Now though there is little distinction between the parts of the mesoderm which occupy the median position of the embryo it will be convenient to regard this mesoderm as consisting of two parts, a median above the neural groove and a lateral portion between it and the somite of each side. For purposes of description I will, therefore, consider the mesoderm as possessing three portions in all: (*a*) somitic, (*b*) subsomitic, and (*c*) median mesoderm. The median mesoderm lies immediately above the median cord of the developing nervous system and while the median cord is giving rise to the transverse commissures of the nervous system the cells of the median mesoderm undergo changes which result in the formation of blood cells (fig. 16, Plate 3). Prominent vacuoles appear in the cells, and as these appear the cells get pushed inwards towards the yolk and thereby liberated. At such a stage a layer of loose spherical vacuolated cells is seen to lie between the developing nerve cord and the yolk. Intersegmentally median mesoderm also gives rise to delicate transverse muscles which are inserted on the ectoderm and pass above the developing nerve cord. These are the only remnants of the connection which existed between mesoderm of the two sides of the body.

That the median mesoderm is a definite formative centre for the production of blood cells is shown by the numerous mitoses which take place in the cells at the time of their liberation. Not all such cells become blood cells. Some pass deeply into the yolk and there disintegrate (fig. 16). The formation of blood cells by disintegration of the median mesoderm heralds the formation of the first part of the hæmocœl. The yolk, till now closely adherent to the mesoderm, moves away from the embryo beneath it to leave a narrow irregular space above the developing nerve-cord. This is the epineural sinus (fig. 17) and into it pass the blood cell products of the median mesoderm. This portion of the hæmocœl remains insignificant in size till the stage shown in fig. 4, when the rudiments of all organs have been laid down. Median sagittal sections of embryos of this stage commonly present the appearance of a longitudinal row of blood cells lying in a narrow space between the yolk and the median ectoderm (fig. 18).

The fate of the median mesoderm is the same for all segments from the mandibular to the posterior abdominal segment. In the thorax the sub-somitic mesoderm is large (fig. 12) and as the limb buds develop, its more ventral part passes down into the hollow of the appendage and gives rise to the intrinsic muscles of the limb. That part which lies closely apposed to the ventral side of the somite gives rise to extrinsic muscles of the leg and to the ventro-longitudinal muscles of the body wall (fig. 19). There has been no attempt made to determine the precise fate of mesoderm in the limbs, beyond the fact that muscle tissue is formed there. Two recognisable extrinsic muscles of the leg arise over the median part of the leg rudiment. Mesoderm cells arrange themselves in curved tracks, as seen in transverse section (fig. 19), and pass outwards from the inner angle of the leg to the body wall immediately ventral to the somite.

The cells become prominently elongated as they arrange themselves in this manner, and at the points of contact with the ectoderm the latter becomes much thickened, owing to modification of its cells to provide muscle attachments. In addition to this transverse muscle is found the elevator, whose main direction is longitudinal, passing from the posterior side of the leg at its junction with the body to the ventral body wall, in front of the leg. Both these muscles, the transverse and longitudinal, arise from mesoderm which lies next to that which passes into the leg itself. The remaining part of the subsomitic mesoderm though originally dorsal to that which gives rise to the extrinsic muscles of the leg becomes enclosed by the transverse leg muscle and develops into the ventral longitudinal muscle of the body wall (fig. 19).

The most dorsal and lateral part of the mesoderm, viz., the somite, occupies, as we have already seen, the hollow formed by the inturning of the body wall. Though the cells forming its walls are arranged radially when seen in transverse section there is not always a sharp line of demarcation between its ventral components and the subsomitic mesoderm already mentioned. As development proceeds it becomes more difficult to determine where somitic and subsomitic mesoderm meet. This difficulty arises chiefly from the fact that the ventral wall of the somite forms a flowing mass of irregularly shaped cells which soon lose to a certain extent their power to take up stain. These cells are the primordia of the fat body and spread in a ventro-median direction over the developing leg muscles whose development we have just considered (fig. 20). The fat cells soon become vacuolated and separated into two masses, one of which lies against the nerve cord, the other remaining in closer contact to the somitic mesoderm, so forming the primordia of the ventral and dorsal fat-bodies respectively (fig. 17).

With the formation of the fat body from the ventral wall of the somite the cavity of the latter disappears or becomes continuous with the epineural sinus. The inner dorsal and outer walls of the somite are now left intact forming a kind of inverted blunt wedge (fig. 17). The outer layer of the somite becomes differentiated into dorso-lateral and dorso-longitudinal muscles of the body wall (fig. 21). Their development is similar to that of the muscles already described except that it occurs later. During this development the body wall is extending up each side of the egg, and it is during the upward growth with which the mesoderm keeps pace, that dorsal muscle differentiation takes place. While the outer layer of the somite has thus lost its regular columnar epithelial form in preparation for its development into somatic muscles, the inner layer still retains its columnar arrangement of cells, and it is only when the endoderm strands have grown backwards above the somites from the mouth region that any change of form is noticed. The first change to be noted is a lifting upwards of the inner wall of the somite towards the endoderm above as if the two were strongly attached to each other. With the appearance of the endoderm strand above the somites the splanchnic layer of mesoderm thus attains a nearly horizontal position and thus widely opens the cœlomic cavity into the hæmocœl below (fig. 17). In their region of communication, however, lies the fat-body often obliterating all sign of a cavity in that place (fig. 19).

The dorsal wall of the somite gives rise to the heart. In stages shown at figs. 17 and 19 the future heart rudiment is not distinguishable, its cells forming part of the normal columnar epithelium of the somite wall. The first sign of its differentiation is to be seen in isolated cells in segmental regions, which appear to have become pushed out of the somite wall between it and the ectoderm (fig. 21). This is the beginning of a general disarrangement of the somite wall here. The somatic muscles have begun to develop against the body wall, above them lie a few irregular placed cells forming the heart rudiment, while on the inner side of these is to be found the splanchnic layer of mesoderm overlain by a few mesenteron cells. The further development of the heart is described in a separate section of this paper.

It must be pointed out that the three regions of mesoderm are not actually defined from one another. The arrangement is purely arbitrary and adopted for purpose of description. In *Pieris,* as the figures show, the whole mesoderm lateral to the nerve cord is very compactly arranged, and many stages of development are incapable of interpretation without reference to earlier and later stages. In the stages described, differentiation is not yet functional, but merely one of arrangement. Thus the muscle tracts are indicated by the arrangement of cells forming them, and not by striations. Similar tracks are formed by growths from the nerve ganglia and only with difficulty picked out from them. Their origins, the one from mesoderm, the other from ventral thickenings of the nerve cord, are the only guides as to the nature of the these embryonic structures.

To summarise the fate of the mesoderm in a thoracic segment :—

(1) Median mesoderm gives rise to blood cells and to a transverse muscle.

(2) Subsomitic mesoderm gives rise to extrinsic and intrinsic muscles of the leg and to ventro-longitudinal muscles of the body wall.

(3) Somitic mesoderm gives rise to visceral muscles, cardioblasts, somatic muscles and fat body.

In all this *Pieris* is remarkably similar to *Apis* as examined by Nelson, 1915, the chief difference lying in the absence of the thoracic legs in the larva and a corresponding absence of mesoderm dealing with intrinsic and extrinsic muscles of the legs. The somite is more distinct in *Pieris* than in *Apis.* In the latter, mesoderm consists of a two-layered sheet of cells passing down each side of the embryo, forming what Nelson calls mesodermal tubes. The inner wall of the mesodermal tube forms visceral muscles and the outer wall forms somatic muscles and diaphragm, while the sheet of mesoderm below the tube and extending downwards towards the middle line forms fat body, ventral muscles and blood cells. Such would seem to be the case in the majority of the specialised insects, such as Hymenoptera (Nelson, 1915), Coleoptera (Hirschler, 1909), and Diptera (Noack, 1901).

An apparently prominent difference is seen in the mesodermal development of Orthoptera. This has been so carefully and beautifully worked out by Wiesmann, 1926, in

Carausius that I will summarise his results rather than those of HEYMONS in his classical account of *Forficula*, etc., 1895. In all Orthoptera the somites are widely open paired sacs, so large that the division of each into its component parts, and its fate, are fairly easy to follow. WIESMANN, 1926, finds that the thoracic cœlom possesses a cavity from the first, which extends ventrally into the limb dorso-laterally along the body-wall and mediolaterally towards the middle line. The ventral and biggest part of the cœlom passes into the limb cavity and forms its intrinsic muscles. The median diverticulum of the cœlom forms the ventral longitudinal muscle, while the lateral cœlomic diverticulum gives rise to fat body and body-wall muscles on its outer side, cardioblasts on its upper side, and the thin connecting layer of cells between the lateral and medial cœlomic diverticula becomes the visceral muscle layer. Further, at the junction between ventral (limb) and dorsal cœlom two pairs of hollow diverticula grow out to form the extrinsic muscles of the leg, namely flexors and extensors.

The first thing to note there is the greater development of cœlomic cavity in the generalised Orthopteron than in Lepidoptera, Hymenoptera and Diptera (in the latter case it is absent), ESCHERICH, 1900; NOACK, 1901, etc. The fact that insects have a hæmocœlic body cavity in the adult is foreshadowed by the reduction of embryonic cœlom. The retention of conspicuous cœlomic cavities in embryos of Orthoptera must be regarded as a vestigial feature. In comparing the differentiation of mesoderm in *Carausius* and *Pieris* WIESMANN'S *Blutzellenlamelle* corresponds exactly to what I have called median *mesoderm*, both giving rise to transverse muscles and to blood cells. The subsomitic mesoderm in *Pieris* corresponds with the ventral medial cœlom and the inner and outer medial diverticula of *Carausius*, which gives rise to intrinsic and extrinsic muscles of the leg and longitudinal muscles of the body wall.

In the dorso-lateral portion of the cœlom of *Carausius* we have obviously the nearly exact counterpart of the somitic portion of mesoderm in *Pieris*. Slight differences, difficult to explain, lie in the different positions from which the fat body arises and the fact that in *Pieris* there is no connection between visceral mesoderm and that which gives rise to the ventral longitudinal muscles. Allowing, however, for the greater development of cœlomic space in *Carausius* than in *Pieris* there is substantial agreement between the accounts given of the fate of the mesoderm in these two insects.

In the fourth and fifth abdominal segments, at the stage shown in fig. 4, certain large spherical cells are seen in the lower part of the inner somite wall (fig. 22). These are the germ cells which, retaining contact with both splanchnic mesoderm and fat cells at this stage, prevent that wide cleavage suffered by the somite in other segments. Surrounding elements soon form an envelope of flattened cells, and these are continued forward as a fine cord on each side of the body as far as the second abdominal segment, where they terminate in contact with the lower side of the cells forming the heart rudiment. I have been unable to determine whether the germ cells differentiate *in situ* or whether they originate from posterior pole cells, which subsequently move forward into this position. No pole cells were seen during blastoderm formation.

5. *The Nervous System.*

(*a*) *Ventral Chain.*—The main features of the development of the nervous system in *Pieris* are easy to follow, but the finer details are more difficult to establish. In the stage in which the mesoderm has just been enclosed by the lateral plates of ectoderm, and while that mesoderm is still piled up into a heap in the middle line, the lateral plates, now joined in the middle line to form the ectoderm, consist of a simple columnar epithelium with nuclei of more or less the same size (fig. 23, Plate 4). A little later the embryo has increased considerably in width, the mesoderm has spread out to the sides preparatory to forming mesodermal somites, and the gastrulation furrow so prominent before has nearly disappeared (fig. 24). To each side of the middle line a great change has taken place, certain cells have enlarged greatly and taken up a position on the inner side of the germ band against the mesoderm. The outer ends of these cells may still remain inserted between the remaining cells of the ectoderm, indicating their origin. The large cells, of which there are at first three rows on each side, are sharply distinguished from the remainder by the fact of their paler-staining cytoplasm and their large vesicular, nearly spherical, nuclei (fig. 24). This is the first step in the differentiation of the ventral nerve cord, and at the stage reached we have recorded the segregation of lateral rows of neuroblasts from the outer smaller dermatoblasts of the covering ectoderm.

Though these lateral neuroblasts play a major part in forming the ventral nerve cord, certain cells which lie between the two lateral neuroblast tracks are also neurogenic, viz., those of the median cord. We therefore have to consider the fate of cells in the lateral and median cords. With the formation of neuroblasts and their sharp delimitation from the dermatoblasts, a deep median invagination develops between the lateral cords, carrying the median cord more deeply into the yolk. Hitherto, the lateral cord neuroblasts of either side formed a more or less flat plate of cells in the horizontal plane, but the deep invagination of the neural groove and the lateral curvature of the embryo alters the position of these rows with regard to one another, so that each set of three rows forms a longitudinal gutter whose base is turned slightly inwards towards the middle line (fig. 12).

The neuroblasts now begin to give rise to small daughter cells on the side next the mesoderm, so that each neuroblast has resting on its inner surface a small daughter cell. Repeated division of the neuroblasts in about the same plane results in the production of a column of daughter cells perpendicular to the inner surface of the parent cell which produced them. Since the neuroblasts of each side are arranged in the form of a longitudinal gutter, and the surfaces from which daughter cells are produced are turned towards a more or less common point, it is in this direction that the daughter cells of the neuroblasts pass, *i.e.*, the columns converge as they pass inwards. The daughter cells do not divide and it follows from this that the oldest daughter cell will be the one nearest the mesoderm, the younger being that one still in contact with the neuroblast. The daughter cells are the future ganglion cells. From the above description it will be seen that the nerve cord is primarily unsegmented, and indeed it certainly appears in *Pieris* as if the segmentation of the nerve cord not only follows that of external segmentation but results from it in a purely mechanical manner.

With the segmentation of the ectoderm the lateral cords become raised up into a series of waves in the intersegmental regions. Segmentally in the longitudinal plane the neuroblasts lie in a furrow the raised edges of which approach the intersegmental region in front and behind (fig. 32, Plate 5). This appears to account for the close collection of the daughter ganglion cells in the middle of each segment. The lateral curvature of the rows of neuroblasts caused a similar concentration towards one circumscribed region and in this manner concentration of ganglion cells is effected in the ventral region of each segment (fig. 31). It is therefore clear that the nervous system develops as two unsegmented cords and only attains its characteristic segmental arrangement after mesoderm and ectoderm have themselves assumed a segmental arrangement.

From the description given the erroneous impression will have been gathered that the lateral nerve cords are distinct from one another. Actually from a very early stage in the development of daughter ganglion cells (fig. 12) the cells of the median cord have taken a part in forming transverse connections between the segmental ganglionic swellings. Median cord cells, *i.e.*, those forming a roof to the neural groove, do not participate in nervous development for the whole length of the embryo. Segmentally a segregation of neuroblasts from dermatoblasts occurs, in the same manner as in the lateral cords (fig. 12). Such neuroblasts give rise to elements forming the transverse commissures joining nerve ganglia of a pair (fig. 30). Intersegmentally the median cord cells take no part in nerve development. In this way a ladder-like ventral nervous chain is formed.

In the region of greatest concentration of ganglion cells, *i.e.*, on the mesodermal side of each developing ganglion, obvious nerve fibres form, by elongation of the cytoplasm of the cells in that region. This fibre region is continuous through each lateral cord. Each ganglion presents the appearance of a cross, formed by nerve fibres passing out in four directions; the anterior and posterior fibres form the intersegmental connectives; those passing directly outwards from the segmental nerves, those passing inwards towards the middle line meet their fellows to form the transverse commissures (fig. 30).

There now occurs a separation of the whole neuroblast layer of each side from the definitive hypodermal cells which lie beneath them. A minute space arises between them and in this space there appears a thin neurilemma. The origin of this is difficult to determine since the cells composing it are ultimately small and few in number. It arises from the outermost products of the neuroblasts, such neurilemma-forming cells becoming elongate and insinuating themselves between the hypodermis and the nerve cord, and also passing above the ganglia underneath the yolk (fig. 25). With the formation of the neurilemma, the space between the nerve cord and the hypodermis widens to form the ventral portion of the hæmocœl continuous with the epineural sinus which lies between the nerve cord and the yolk (fig. 22).

At the end of the embryonic period no neuroblasts are distinguishable. Whether this is due to their taking on the size and function of normal ganglion cells or to their disappearance by degeneration is uncertain. Signs of degeneration are to be seen in the ventral regions of nerve ganglia in fully or nearly fully formed embryos, and these may represent disappearance phases of neuroblasts, as found in the Orthoptera by WHEELER (1893) and in Hymenoptera by NELSON (1915).

(*b*) *Brain.*—The nerve tissue develops in the head as in the body, *i.e.*, from neuroblasts which segregate from dermatoblasts and which by successive mitosis give rise to ganglion cells. There only remains to describe the formation of the separate parts of the brain. At all early stages of development, the cephalic lobes, which are markedly wider than body segments, show a marked constriction at each side. This constriction is found some little distance in front of the bases of the antennæ and marks off the protocerebral from the antennary or deutocerebral segment (fig. 26). In the protocerebral area we find the greatest development of nerve cells from a large area of neuroblasts, which extend as far as the front border of the embryo and behind are continuous with the neuroblasts of the antennal segment (fig. 27). Medially the neuroblasts in this region are only separated from one another by the bases of the labral lobes and the upper surface of the stomodæum. The antennary segment is a little narrower than the protocerebral and the area which gives rise to the deutocerebrum lies immediately in front of the base of the antenna. Behind the latter appendage lies the tritocerebrum, which is derived from the region postero-laterally to the mouth. When the premandibular ectoderm has given rise to the trito-cerebral neuroblasts it loses its distinctness as a segment and is no longer distinguishable from the hypodermis forming the posterior walls of the stomodæum. In this I support TOYAMA (1902). The three pairs of ganglia forming the brain therefore clearly indicate three anterior segments of the head, the tritocerebral ganglia being continuous with the first or mandibular ganglia of the subœsophageal nerve mass.

A transverse commissure, serially homologous with those of the body region, passes under the œsophagus and connects the tritocerebra of the two sides (fig. 28). It appears as an inward-turning branch, which separates from the para-œsophageal connectives. HEYMONS (1895) described the development of a similar commissure in *Forficula* and states that it arises from the median cord of the third head segment. In *Pieris* such a commissure develops from the median inner cells of the tritocerebral ganglia (fig. 28). The presence of such a subœsophageal commissure clearly indicates that morphologically the third head segment is post-oral and not pre-oral as stated by STRINDBERG (1913).

The protocerebral ganglia only become connected by a supra-œsophageal commissure after the backward passage of the mouth. With this movement the labral lobes unite with one another and passing back, with the mouth, leave behind them a thin-walled hypodermis covering the fore-brain. This hypodermis is at first deeply grooved in the middle line, and it is from the cells forming this groove that the supra-œsophageal commissure is formed, the inner edges of the protocerebral lobes keeping in contact with this portion of the hypodermis till the commissure is formed. When this is effected the brain is completely separated from the hypodermis from which it arose. Fig. 29 shows the supra-œsophageal commissure in process of differentiation fron the hypodermis bounding this groove.

The development of the nervous system in insects has been investigated by a number of prominent workers, among whom may be mentioned WHEELER (1893), HEYMONS (1895) in Orthoptera and Dermaptera, LECAILLON (1898) in Coleoptera, CARRIÈRE and BÜRGER (1898) and NELSON (1915) in Hymenoptera, HIRSCHLER (1909) in Coleoptera, ESCHERICH (1902) in Diptera. There appears to be no modern account of nerve development in Lepidoptera. HATSCHEK (1877) gave a short account of nerve development in *Bombyx*. My findings agree in the main with his account as with those of the above-mentioned workers, and no useful purpose is effected by describing this system in further detail.

It should be noted that ESCHERICH (1902) found in *Lucilia* that the whole of the median cord becomes split off from the ectoderm by closure of the primitive or neural groove. Intersegmentally lateral strands pass out to the tracheal invaginations as sympathetic nerves, and according to this author the ventral nervous system has a double origin, the voluntary system arising from the lateral cords, the sympathetic system from the median cord, the two joining secondarily with each other. In fully formed embryos such sympathetic nerves can be found in *Pieris*, taking their origin from the median line position of the ventral nerve cord, but their precise development is obscure.

(*c*) *Stomatogastric System*.—This arises at two points in the dorsal wall of the stomodæum, the one behind the other in the median line (fig. 27). Cells in these two centres become pale and rounded (as is the case in the differentiation of nerve cells from primitive ectoderm to form the ventral cord). They then extend upwards from the wall of the stomodæum to lie on the surface of the latter. For a time, even after the two ganglia so formed (the frontal and stomatogastric) have united to form the stomatogastric nerve, these two centres retain their connection with the layer of cells from which they arose, some obvious nerve cells remaining embedded in the epithelium of the stomodæum (fig. 32).

The stomatogastric nerve is produced by extension of nerve cells from the two ganglia along the roof of the stomodæum. From the frontal ganglion, which eventually is fairly large and round and situated within the labrum, lateral nerves grow out and downwards round each side of the pharynx and run into the front part of the circum-œsophageal commissure. HATSCHEK in his embryological study of *Bombyx* failed to note the real origin of the sympathetic nerves, but showed them lying in the œsophageal musculature.

6. *The Alimentary Canal.*

The alimentary canal develops in three sections, the fore-, mid- and hind-gut. By far the greatest interest is attached to the story of the development of the mid-gut. In the last half-century numerous investigations have been made, and there is perhaps no subject in morphology on which such difference of opinion has been expressed. The peculiarities of development of the mid-gut have made it difficult to bring it into line with the germ layer theory. Attempts have been continually made to derive the condition found in the insect egg from the typical invertebrate gastrula and to homologise the mesenteron rudiments with the endoderm of such a gastrula. From a perusal of the appropriate literature it would appear that lack of agreement arises more from differences of interpretation than from actual differences in developmental process.

(*a*) *Mid-gut.*—I have already pointed out (Eastham, 1927) that anteriorly and posteriorly in the regions of future mouth and anus, cell masses are proliferated inwards from the germ band towards the yolk in front of and behind the main mesoderm mass respectively. The formation of these cell masses differs from that of the mesoderm, an early indication of the different fates destined for these masses. That cell mass which is formed in the oral region, which I have already called the anterior endoderm rudiment, arises at the beginning of the gastrulation process and the corresponding posterior endoderm rudiment arises later—in fact its development coincides with the completion of gastrulation. It follows that at the end of gastrulation we shall find the anterior endoderm rudiment much bigger than the posterior rudiment. The stomodæum and proctodæum now develop as invaginations in the ectoderm, covering the endoderm rudiments. In some cases the invagination forming the stomodæum occurs before proliferation of endoderm cells has ceased. The endoderm then *appears* to develop from the stomodæal ectoderm. This is usually the case with the posterior endoderm rudiment, proctodæal development following rapidly on endoderm formation. In the majority of cases, however, the anterior mesenteron rudiment becomes quite sharply defined, while the stomodæum is the shallowest of depressions (fig. 33, *a*).

The anterior rudiment, as previously described and figured (Eastham, 1927) has at first a rounded contour, but when the stomodæum begins to develop it proceeds to spread towards its circumference between the embryo and the yolk (fig. 33, *a*). Such circumferential spreading accompanied by prominent mitotic divisions of the cells gives rise to the mesoderm of the first three cephalic segments. Not only does the rudiment spread outwards in the above described fashion but cells proceed from its inner side and penetrate between the yolk cells (fig. 33, *a*). These do so in three principal directions: Those from the middle of the inner surface pass directly into the yolk and separate as they do so; some of these form blood cells, others degenerate soon after taking up their new position. At the two lateral angles of the endoderm mass, however, the cells elongate to form two tongues of cells, each passing laterally into the yolk. These are the true endodermal strands (figs. 33, *a*, 35 and 33). The cells forming these strands undergo mitosis as they proceed under the yolk in a gentle curve roughly parallel with the post-oral part of the germ band and form a chain of cells, one cell thick, above the mesoblastic somites in the anterior body segments. Figs. 19 and 20 are transverse sections showing endoderm strands in position.

In the formation of these endoderm strands mitosis takes place in the plane transverse to the direction in which the cells are moving (fig. 33), so that the initial loose and somewhat disconnected trail of cells becomes more continuous and better defined. We now have two strands of endoderm cells passing backwards from the point of origin of the endoderm against the blind end of the stomodæum. From these two endodermal strands and the corresponding ones from the posterior end of the embryo the whole of the mid-gut develops, first of all by increase of cells to connect the two strands together beneath the yolk, so forming an endodermal gutter holding the yolk (figs. 32 and 22) and later by upward growth of the walls of this gutter to form a complete tube. The first place in which the median ventral growth of the mid-gut takes place is in the region next the stomodæum (fig. 32). At this point the endodermal strands are already connected by the original endodermal mass. An increase in the number of cells takes place here so as to extend the ventral endoderm further and further back. During this process the cells forming the blind end of the stomodæum consist of a very thin layer, and one no longer sees a sharp line of demarcation of stomodæum from endoderm. Whether this means that addition of cells from the stomodæum to the mid-gut is taking place or not, is difficult to determine.

Mitosis is commonly seen in the mid-gut rudiment and this appears to account for the main growth of the mid-gut. The development of the mid-gut from the proctodæal end is very similar to that from the anterior end. The main difference lies in the fact that the proctodæum begins to form, while the posterior endoderm mass is still small. This entails a more prominent proliferation of cells from the blind end of the proctodæum. In other words, the development of the proctodæum coming so soon after the beginning of the development of the posterior endoderm mass, the formative area of the posterior endoderm rudiment is carried inwards to the end of the proctodæum. It is this fact which I believe had led many insect embryologists to the conclusion that the midgut is ectodermal in origin. To this point I shall refer later. The development of the mid-gut from this region is from now onwards very similar to that already related concerning the growth from the anterior end. Two strands pass backwards under the outer edges of the proctodæum, until they meet the tenth abdominal somite on each side (fig. 36, Plate 6). They then pass forwards above the remaining somites just as the anterior strands pass backwards. Anterior and posterior strands meet at each side and soon a complete endodermal groove carrying the yolk above it is formed (figs. 22 and 37). The continued growth upwards and inwards of the sides of this endodermal groove results in the completion of the mid-gut in the form of a tube, holding within it some remnant of the yolk, and lying between the still blind ends of the stomodæum and proctodæum.

(*b*) *Fore-gut.*—The stomodæal development is simple. Its position has already been noted. It is at first a simple invagination which is directed towards the anterior end of the egg, *i.e.*, obliquely to the surface of the ectoderm; then as the preoral ectoderm increases in extent the stomodæum becomes more definitely directed at right angles to the ectoderm at its borders (figs. 38 and 34). The inner end of the stomodæal cavity now changes in shape; the floor or end wall of the stomodæum becomes thin, and where it joins the lateral walls of the stomodæum becomes reflected forwards towards the mouth, so as to form a short jacket round the stomodæum in this region (figs. 34 and 38). In this way the whole stomodæum becomes rather mushroom-shaped, the upper surface of the "mushroom" which forms the curtain separating the fore-gut from the mid-gut, being extremely thin. A sagittal section of the stomodæum shows a thick dorsal wall, a thinner ventral wall and an extremely thin wall forming its inner end. At an early stage the stomodæum is surrounded by mesoderm which belongs to the three first segments of the head. Both preoral and antennary mesoderm eventually come to lie on the dorsal wall of the stomodæum. The premandibular mesoderm remains closely attached to the ventral wall of the stomodæum and gives rise to the subœsophageal body. The preoral mesoderm becomes developed into the stomodæal musculature. Since it occupies from the beginning a dorsal or anterior position to the mouth one can readily understand why there appears no musculature on the ventral side of the stomodæum but a prominent mass of mesoderm on the dorsal side (fig. 38). The preoral mesoderm at that stage has not yet enveloped the stomodæum and only later does the complete investment take place. Beneath the stomodæum (fig. 34) is the premandibular mesoderm in close association with numerous blood corpuscles. It is seen to be in close contact with the endoderm rudiment and retains this connection as the stomodæum deepens. In this way the premandibular mesoderm is carried deeply with the developing stomodæum into the yolk region and far from its place of origin. During this change of position the cells composing it become enlarged and much vacuolated, and in the last stage of development they are seen as a transverse row or two of large spherical vacuolated cells beneath the connection of endoderm and stomodæum (figs. 38 and 39). It is then known as the subœsophageal body.

The significance of these cells is unknown; Toyama (1902) in *Bombyx* noticed mitosis occurring in them and from the close proximity of large numbers of blood cells, concluded that it was from here that the main mass of blood cells arose. I cannot confirm this in *Pieris*. The blood cells are certainly closely congregated in this region but there is nothing in my preparations to show that they originate in the subœsophageal body. In *Pieris*, the only noticeable change in these cells is in size and not in number; I have seen no mitotic figures in them. Fig. 34 shows a row of blood corpuscles behind the subœsophageal body, but such blood cells are found along the mid-ventral line of the whole embryo and originate in the mesoderm which connects the somites of the two sides together.

(*c*) *Hind-gut.*—The development of the proctodæum is so similar to that of the stomodæum that little need be said. It is an invagination of the ectoderm which occurs in the terminal part of the embryonic rudiment behind the eleventh abdominal somite. In fig. 14 we have a sagittal section of an embryo before the proctodæum has begun to develop. The cells forming the posterior endoderm rudiment are in the posterior recurved part of the embryo. The somites are not yet clearly formed but the heaped up mass in each segment is obviously an incipient somite and it is behind the last of these that the proctodæum develops, the invagination occurring in a dorsal direction. Its connection with the posterior endoderm rudiment has already been commented upon. From its walls three pairs of tube-like outgrowths develop. These are the six malpighian tubules (fig. 40). When these first appear, they arise from the proctodæal wall near its blind end. Then follows a great increase in length of the proctodæum from the point of origin of the tubules inwards, so as to leave the malpighian tubule connection with the gut quite far behind and near the anus (fig. 41). The region in front of the malpighian tubules constitutes the colon and is separated from the hinder portion or rectum by a sphincter muscle.

(*d*) *Discussion on Endoderm Formation.*—In the attempts to harmonise the peculiar features of insect development with those of typical gastrulation, the development of the mid-gut has always played a prominent part. To the various theories on the origin of the endoderm I have already referred (EASTHAM, 1927). NELSON, 1915, gives an admirable summary of the subject and I refer the readers to his account and to my review of this subject (EASTHAM, 1930). The position can be reduced to three different expressions of opinion. One view adopted is that true endoderm is present and may be derived from yolk cells as for the Odonata (TSCHUPROFF, 1903). (Many authors might be quoted here on Lepidoptera, Hymenoptera, Orthoptera, Hemiptera, but their work has since been called in question by recent investigators or has been contradicted by later work prosecuted by themselves.) Others, still believing in true endoderm, express the opinion that the endoderm is derived from anterior and posterior proliferating areas of the blastoderm distinct from the mesoderm (*vide* NELSON, 1915, in *Apis*, NOACK, 1901, in *Calliphora*, CARRIÈRE and BÜRGER, 1898, in *Chalicodoma* and *Tenebrio*), or that the endoderm is derived from the two ends of the lower layer (mesentoderm) (*vide* WHEELER, 1889, in *Blatta*, SCHWANGART, 1904, in *Lepidoptera*, etc.), or that the endoderm is derived from the Splanchnic layer of the mesoderm, *e.g.*, HEIDER, 1885, in *Coleoptera*.

The third main opinion is that true endoderm takes no part in the formation of the embryo but is purely embryonic and transitory, and is represented in the egg by yolk cells which disintegrate. HEYMONS (1894 and 1895) is the great champion of this view, and he and his followers derive the mid-gut from cell proliferations at the blind ends of the stomodæum and proctodæum. On this view the mid-gut is ectodermal, and in consequence no true mesenteron is present in insects (see MANSOUR, 1927). In view of the more recent work of PHILIPTSCHENKO (1912) clearly disproving the origin of endoderm from yolk cells in Apterygote insects, and in view also of the close similarity in the origin of endoderm from separate anterior and posterior cell masses and from anterior and posterior ends of the mesoderm, it will be seen that the matter really rests between the question of a bipolar endoderm and a mid-gut derived from the blind ends of the stomodæum and proctodæum. In a short appendix to my earlier paper, 1927, I commented on the possibility of bringing these two views into harmony, and since then I have noticed a paper by Nusbaum and Fulinski, 1909, where the same view is expressed at greater length.

In insects the mid-gut is surely homologous throughout, and any difference of developmental process must not be too much stressed as indicative of lack of homology. Convincing accounts for instance of these by Nelson on *Apis* (1915) and Mansour on *Calandra* (1927) must be accepted. Nelson clearly points to a bipolar origin of the mid-gut which has nothing to do with the stomodæum and proctodæum. Mansour, on the other hand, just as clearly shows stomodæal and proctodæal (therefore ectodermal) origin of the mid-gut. It would be ridiculous to assume from this that the mid-guts of the two subjects for investigation quoted are not homologous structures, and yet that is the conclusion to which we are driven if we accept each author's view and attempt to keep rigidly to the germ-layer theory.

The explanation seems to lie in the fact that in different types of insect embryos, the *anlagen* of the mid-gut arise at different times relative to the stomodæum and proctodæum. This view, clearly expounded by Nusbaum and Fulinski (1909) is that there are no fewer than seven different ways in which the mid-gut develops, only differing from one another in the earliness or lateness of development as compared with the development of the stomodæum and proctodæum. The following is a summary taken from Nusbaum and Fulinski (1909) :—

Type I.—Endoderm rudiments are first cut off from the germ band. This is followed by the invagination of stomodæum and proctodæum carrying the endoderm into the yolk. Noack, 1901, in *Muscids* ; Hirschler, 1909, in *Donacia.*

Type II.—Endoderm rudiments formed from the germ band, but the stomodæum invaginates before these are cut off from the ectoderm. Karawaiew, 1893, in *Pyrrhocoris.*

Type III.—In anterior region as Type II. In posterior region as Type I. Nusbaum and Fulinski, 1909, in *Gryllotalpa.*

Type IV.—Stomodæum and proctodæum develop immediately in front of and behind the endoderm rudiments respectively. Nusbaum and Fulinski, 1906, in *Phyllodromia.*

Type V.—Stomodæum, proctodæum and endoderm rudiments develop concurrently and lower layer contributes to mid-gut formation. Hirschler, 1909, *a*, in *Gasteroidea.*

Type VI.—As in Type V, but no lower layer contributing to endoderm formation. Carrière and Bürger, 1898, in *Chalicodoma.*

Type VII.—Growth areas of endoderm rudiments remain dormant till the stomodæum and proctodæum appear. Heymons, 1895, in *Forficula.*

From a consideration of these various methods of gut development we can see, as already pointed out by me (EASTHAM, 1927), that there can no longer be grounds for continuing the controversy any further. There is no essential difference between a mid-gut which develops from rudiments arising directly from the germ band and one which arises from anterior and posterior cell proliferations at the blind ends of the stomodæum. In the former case cell proliferation to form endoderm begins before the stomodæal invagination, whereas in the latter case endodermal proliferation begins later, after the stomodæal development has occurred.

It is not surprising to find developmental processes intermediate between these two. Such is the case in *Pieris* where it is seen that anterior endoderm is proliferated from the germ band and may continue to be so formed during stomodæal development. It might be asked why there is no agreement in this matter between the development of, say, *Pieris* and *Bombyx,* both belonging to the same order. TOYAMA, in *Bombyx,* describes the development of an oral cell mass—admittedly the same as the anterior endoderm rudiment in *Pieris.* This mass in *Bombyx* reaches a maximum development and subsequently disappears and takes no part in mid-gut formation, whereas the corresponding mass does contribute to the mid-gut in *Pieris.* The development of *Pieris* is rapid—can be completed in 3 days—whereas that of *Bombyx* under the conditions employed by TOYAMA is slow. Eggs laid in July reach the stage of gastrulation in December. The oral cell mass is now present and the egg goes into a resting stage till the end of January. Though TOYAMA does not make this point clear, it seems highly probable that this hiatus in the developmental process may account for discontinuity between the oral cell mass and the proliferation which arises at the end of the stomodæum and gives rise to the anterior endodermal strands. This again may account for the different conclusions of STRINDBERG (1913) on this point in the development of *Bombyx* ; for he discredits TOYAMA's conclusions and declares that in *Bombyx* the mid-gut does not develop from the stomodæum and proctodæum but from proliferations in the germ band. RIZZI (1912) maintains that in *Bombyx* the mid-gut arises from ectodermal proliferations which penetrate the underlying mesoderm. This is a very different state of affairs from that shown in fig. 33, *a.* Careful study of this point does not enable me to confirm this author's account.

7. *The Heart.*

After the derangement of cells in the dorsal wall of the somites, this being the first sign of cardioblast formation already mentioned (p. 11), these same cells take on an obliquity, their outer ends turning outwards towards the hypodermis some little distance below its dorsal edge. The hypodermal cells in this region become thickened, and slender extensions of them pass onwards and make contact with the outermost cardioblasts (figs. 17 and 21). This connection is maintained throughout the whole period of heart development. The corresponding connection between the innermost cardioblasts and the splanchnic mesoblast is retained until the heart rudiment meets its fellows on the opposite side.

The condition of the embryo at this stage (fig. 17) as seen in transverse section is one in which the lateral body wall extends a little more than halfway up one side. The heart rudiment of each side is slung like a hammock between the more dorsal region of the hypodermis and the splanchnic mesoderm. Between this and the nerve cord are to be found the developing muscles and fat cells. The whole system of organ *anlagen* now grows in a dorsal direction at each side. The body wall grows upwards on each side, to meet in the mid-dorsal line to enclose a portion of the yolk (fig. 37). The somatic muscles accommodate themselves to this change by increasing in length. On an arc of a circle parallel to that traversed by the body wall in its dorsal course, the heart rudiment, concave on its upper surface, moves dorsalwards. It is obvious that the heart rudiment on each side must pass through a position in which its concave surface (originally upper) faces directly inwards towards its fellow. This condition is seen in figs. 39 and 37, in which the developing structure is seen dorsal to the proctodæum and incomplete hind-gut respectively.

It is at this stage that the heart severs its connection with the alimentary canal and by an approximation of the two rudiments to one another, which is assisted by the contraction of the till now thin dorsal hypodermis, an inverted gutter-like structure is formed in the mid-dorsal line between the alimentary canal and the dorsal body wall (fig. 42, Plate 7). This quickly becomes converted into the tubular heart characteristic of insects by the final junction of the two edges of the inverted gutter. It is only in the final region of closure of the embryo from the yolk that the heart completes its development before the body wall.

The pericardial diaphragm is formed from cells of the somite wall which lie immediately beneath the original cardioblasts. Their precise development is obscure but they are clearly seen in the stage shown in fig. 37, passing from beneath the heart rudiment to the hypodermis. Though there are always blood cells associated with the developing heart, I can find no evidence for the statement that these have a common origin with the cardioblasts. The heart extends as far forward as the first thoracic segment above the œsophageal valve and there it meets and fuses with the posterior end of the cephalic aorta which develops from the antennary mesoderm. It is while the anterior end of the heart is in the form of an inverted gutter that the cephalic aorta makes connection with it.

8. *The Aorta.*

The antennal mesoderm, from which originates the cephalic aorta, early divides into two parts, an outer which clings to the growing appendage and an inner larger part, which passes in towards the stomodæum as a solid mass of cell (fig. 26). These cells are easily recognisable, lying dorsal to the stomodæum and to each side of the frontal ganglion. By the time the latter ganglion has become separated from the stomodæal wall, cells from each antennal mesoderm mass have passed in an anterior direction to join the hypodermis so as to enclose the frontal ganglion between two vertical walls.

In the region where the frontal ganglion passes into the recurrent nerve, the antennary mesoderm embraces the stomodæal wall at each side and dorsally, the recurrent nerve lying between the mesoderm of the two sides above the stomodæum (fig. 33). The antennary mesoderm cells now creep over the nerve to enclose it (fig. 43) passing above it and insinuating themselves between the nerve and the stomodæum. In this way a tube is formed above the stomodæum and enclosing the recurrent nerve. The cavity enclosing this nerve now enlarges and the antennary mesoderm becomes less compact (fig. 44). The antennary mesoderm now spreads down each side of the stomodæum and at the same time the cavity enclosing the recurrent nerve extends down each side so as to embrace the stomodæum on all sides except the ventral (fig. 45). Thus the aorta now lies like a collapsed tube over the top and sides of the stomodæum.

Behind the subœsophageal commissure the aorta is restricted to the form of a fine tube passing back towards the œsophageal valve region (fig. 46). The aorta is open to the body cavity both in front and behind. In front the opening is permanent, the dorsal aorta wall extending as a fine sheet of cells further forward than does its ventral wall (fig. 47). The posterior end is only open as long as it is unconnected with the heart.

No comment is needed on the subject of heart development. My findings agree with those of all other researchers consulted, of whom the most recent are NELSON (1915) and WIESMANN (1926). Most authors are in agreement as to the antennal mesoderm forming the aorta. An outstanding exception is HIRSCHLER (1909), who in *Donacia* finds that the intercalary mesoderm gives rise to this organ. HEYMONS (1895), STRINDBERG (1913), NELSON (1915), WIESMANN (1926) find that the antennal somites meeting over the œsophagus enclose a space, so forming the aorta. NELSON (1915) in *Apis*, and STRINDBERG (1913) in *Eutermes*, report widely open antennal somites which meet and surround the œsophagus in forming the heart. Only in these minor details do their accounts differ from mine.

9. *The Tracheal System.*

This system develops from spiracular invaginations anterior and lateral to the paired appendages in the pro- and meta-thoracic and the first eight abdominal segments (fig. 5). The meta-thoracic spiracle closes early in development and is not seen in an embryo of the stage shown in fig. 6. Each invagination is at first a simple blind tube ending in flattened extensions which pass upwards within the lateral muscles of the body wall and inwards towards the nerve cord (fig. 20). From the inner end of each spiracular invagination growth in a longitudinal direction results in the formation of a lateral longitudinal trunk and from the junctions of the latter with spiracular trunk there pass three tracheæ. Of these one passes upwards to the dorso-lateral region of the body wall and its muscles, a second passes ventralwards to the body wall, and the third inwards to the alimentary canal. It is of interest to notice that TOYAMA (1902) found 10 pairs of abdominal spiracles in the embryo of *Bombyx* (see IMMS, 1925, fig. 170). Of these the two posterior pairs close before hatching. I am unable to confirm this in *Pieris*.

10. *Blood and other Cells released into the Yolk.*

From the embryo there is for a time a constant release of isolated cells into the yolk. These are liberated from the whole length of the mesoderm and most markedly from the anterior mesenteron rudiment. This fact has already been referred to on p. 17. When the mesoderm is first congregated into paired segmental masses, but not yet forming definite somites, certain cells, those nearest the yolk, are set free, and for a time they form an indiscreet sheet of cells partially separating the embryo from the yolk (figs. 16 and 18). Sagittal sections therefore show a loose line of irregularly shaped cells lying on the inner side of the mesoderm. Those which originate from the mesoderm appear to retain this position, but those derived from the mesenteron rudiment pass deeply into the yolk.

Such liberated cells, at first resembling normal insect blood cells, quickly take on a characteristic appearance. As they pass into the yolk they become spherical and highly vacuolated, each possessing three or four spherical vacuoles. The yolk in which they lie, being that adjacent to the embryo, also quickly changes from a compact to a vacuolated and fluid state. From this it would be reasonable to assume that they not only serve as digestive agents, whereby the embryo can more easily assimilate its food store, but as organs which reduce the yolk to a more fluid state so enabling the embryo the more easily to effect those turning movements known as blastokinesis.

Such cells are not long-lived and are not to be confused with the true blood cells, which appear later. As the liberated cells enter the yolk the nuclei of the latter begin to lose to a considerable degree their characteristic appearance. From now onwards yolk nuclei become shrunken, the chromatin becomes aggregated into irregular patches and the beautiful streaming strands of cytoplasm, which passed out from the nuclei in early stages, now become contracted and lack differentiation (fig. 35). The cells passing from the anterior mesenteron rudiment pass into the narrow spaces between the large yolk cells, so that for a time the latter are enmeshed by them (fig. 35). The lateral masses of mesoderm (future somite) do not contribute to these phagocytic cells. Those of mesodermal origin arise from the mesoderm strands which connect the somites of a pair, and this region continues to liberate such cells after the formation of somites and up to the time when the endoderm strands are lying longitudinally above the somites. When the endoderm strands are well formed and continuous along the embryo, no further cells are released.

In the partial dissipation of median mesoderm to form blood cells increase of cells by mitosis occurs. The layer becomes broken up into a disconnected sheet of cells (fig. 16). Certain cells at once wander into the yolk rendering it apparently more fluid. Such migrant cells soon disintegrate, others remain in the narrow space between the yolk and the germ band. They soon lose their amœboid form (fig. 16), and become spherical developing large vacuoles, as many as three or four occurring in a single cell which ultimately seem to join together to form a large spherical space nearly as big as the cell itself. The commonest state in which one finds blood cells is that of the form of a signet ring, the cytoplasm being extremely attenuated except where it surrounds the nucleus (fig. 18).

11. *Œnocytes.*

These are formed as groups of large cells in the hypodermis lying behind each of the abdominal spiracles. A gradual segregation of hypodermal cells and œnocytes takes place, the latter becoming large and round and passing inwards as a spherical mass of cells behind the spiracle (fig. 48). Though the cells retain their contiguity they become less closely arranged and persist as a rather loose mass of cells in close association with the hypodermis and the spiracle (fig. 49).

12. *The Amnion.*

From the time when the amnion and serosa become separated by a small portion of yolk till the end of embryonic development, no further changes were noted in the serosa. In an embryo at gastrulation (fig. 1) the embryo is in contact with the yolk over the whole of its inner surface. As the body wall grows upwards at each side, the area of contact with the yolk becomes gradually diminished. Also as earlier blastokinesis takes place, whereby the embryo straightens out, a portion of that yolk which lay in the curvature of the embryo becomes lifted up and removed from the embryo towards the space between the amnion and serosa.

In the abdominal region the first segment to be completed is the tenth. It will be seen that in growing upwards towards the mid-dorsal line the edge of the body wall carries with it the amnion to which it is connected. A fusion between body walls of the two sides also involves the fusion of their two amnions, and for a time a rather thick plate of tissue remains above the segment, the dorsal body wall and fused amnions forming a compact mass. By the time this condition is arrived at in the segment in front, the amnion has become completely severed from the body wall and these two tissues now lie closely opposed to one another, as thin flat sheets of cells (fig. 37). The prothoracic segment is the position where the embryo finally severs its connection with the amnion (fig. 6). When dorsal closure of the embryo is finally effected the amnion remains as a continuous thin sheet separated from the embryo by a fluid-filled space, the amniotic cavity. It is thus that the embryo severs its connection from that residual yolk which lies between the amnions and the outer serosa.

13. *The Corpora Allata.*

These are solid masses of cells arising by invagination from the same ingrowth as the mandibular apodeme. They early become separated from the latter and lie against the side of the œsophagus behind the brain (fig. 46). Ito (1918) has made an examination of these structures in several Lepidoptera, in which he finds them to function as glands in the adult, after becoming enlarged during metamorphosis. Their development in *Pieris* agrees in every respect with the findings of ITO. In *Dermaptera* HEYMONS (1895) finds that they belong to the maxillary segment. In *Apis*, however, NELSON (1915) ascribes them to the mandibles, but his suggestion that the corpora allata and mandibular glands are homologous is obviously wrong, since in *Pieris* these two organs are both present ; more morphological and physiological research is needed on these interesting organs before any adequate conclusions can be arrived at concerning their morphological significance and their function.

14. *The Development of the Head.*

Any conception of the constitution of the head of Arthropods should essentially be based on embryological investigation. The criteria indicative of segments, namely appendages, mesoblastic somites and neuromeres, must be critically examined at all stages of development. This is particularly important, in that an appendage may be purely embryonic, mesoblastic somite formation may be reduced to a minimum, and neuromeres may differ in size from one another or may be closely applied together. Each such condition rendering head segmentation a subject difficult of interpretation has been encountered in the study of *Pieris*. Thus premandibular appendages are extremely minute and are early absorbed into the body of the embryo. It is only in the mandibular, maxillary and labial segments that mesoblastic somites are readily definable (fig. 50, Plate 8). The three segments anterior to these possess mesoderm which arises in a different manner from that of the region behind them, and in these segments only, mesodermal masses rather than definite somites are differentiated. The tritocerebral neuromere is small and develops in close contact with the deutocerebral neuromere. Added to these are the further difficulties presented by the great curvature of the embryonic head and the fact that external segmentation manifests itself before the mesoderm has taken on a metameric arrangement.

(*a*) *Appendages.*—The antennæ, mandibles, maxillæ and labium develop in the usual manner as paired hollow evaginations of the ectoderm beneath their respective mesoderm masses and lateral to their neuromeres. They are from their first appearance easily recognised and are all primarily postoral in position. As soon as the embryo undergoes the anterior dorsal curvature already described (EASTHAM, 1927) an anterior region occupying a dorsal position in the egg (fig. 16, of that paper) develops extensive lateral outgrowths prominently incurved at the lateral and anterior borders. The embryo at this point is nearly twice as wide as in other regions, and the two prominent lateral lobes, constituting the rudiments of the proto- and deutocerebral lobes of the brain, are seen at first to be separated by a median ventral groove in direct line with the neural groove of the body behind. From the posterior side of each such lobe there soon grows out a rounded extension of the ectoderm, the antenna. Behind the antennal buds the embryo becomes rapidly narrower, and it is in this region of narrowing that the tritocerebral or premandibular appendages are found (fig. 7). These are so short-lived that they can only be found in embryos of an age at which antennal rudiments are first forming. They are median and posterior in position to the antennæ and lie immediately behind the shallow invagination of the future mouth.

The next three pairs of appendages, mandibles, maxillæ and labium have a typical development, and reference need only be made to the fact that they occupy the vertically curved part of the head, the mandibles having a dorsal and the labium a ventral position in the egg (fig. 2). A transverse section through this region therefore passes through all three segments (fig. 50). If one examines embryos of the stage shown in fig. 2 (at this stage premandibular appendages have disappeared) two distinct sets of structures are seen in front of the mouth. Projecting forwards and occupying the median groove which separates the protocerebral lobes from one another is a pair of prominent lobes, whose bases meet in the middle line and are continuous laterally with the protocerebral lobes of the future brain. Their bases are separated by a distance of some 30 μ from the mouth. The latter is further overhung on its anterior side by a median unpaired lobe, quite distinct from the above-mentioned structures. Fig. 51 is a drawing of a transverse section through the procephalic lobes, with the pair of median appendages between them. Fig. 52 is taken from the same series of sections and is five sections removed from the former.

Before deciding as to the significance of these lobes, we must trace their development. The anterior lobes are the primordia of the labrum, and in *Pieris* there is no doubt as to the bifid nature of this organ at its first appearance (fig. 51). Each half of the embryonic labrum is a hollow extension of the body wall and into it passes a portion of the preoral mesoderm. The median lobe immediately overhanging the mouth (fig. 52) constitutes the epipharynx, and it is interesting to note that this also has its origin in a pair of minute lobes forming an anterior border to the mouth and lying quite clear of the larger labral lobes in front of them (fig. 53). Its double nature is of short duration and as the stomodæum develops deeper into the yolk the epipharynx is rapidly converted into a single median lobe (figs. 54 and 52) from which all trace of its paired origin has entirely passed. Into the epipharyngeal lobe there also passes a portion of preoral mesoderm. Whether it is reasonable to regard labrum and epipharynx as the homologues of true appendages will depend more on any association between them and mesoderm and neuromeres than on their superficial resemblance to true embryonic limbs. They will, therefore, be considered more fully after the head mesoderm has been described. Their further development consists more of movement than of change of form. The labral lobes moving backwards, gradually approach the anterior border of the mouth and in so doing come to lie against the epipharynx. With this they fuse and the epipharynx thus comes to occupy a position under the labrum proper and facing the mouth. In this way is formed the labrum-epipharynx of the caterpillar. The latter, it may be mentioned, possesses a slightly bifid labrum reminiscent of the double origin of this organ.

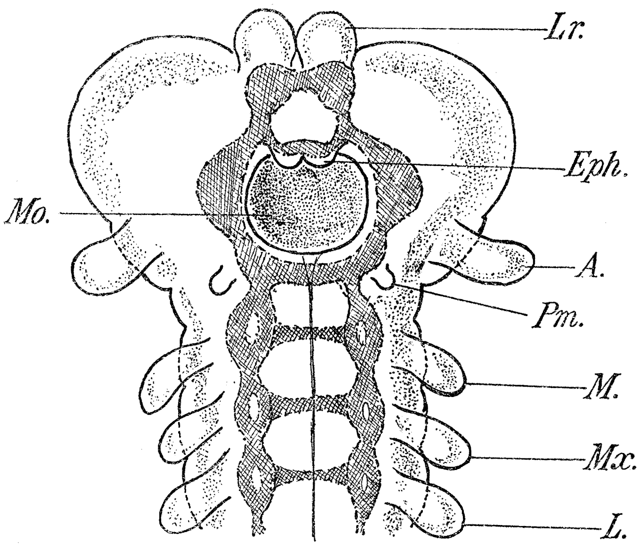
(*b*) *Mesoderm*.—It has already been pointed out (Eastham, 1927) that anterior to the region concerned with mesoderm formation by overgrowth and invagination, a region of proliferation is set up from which develops a mass of cells, the anterior mesenteron rudiment. The first three pairs of mesoblastic somites behind this are those of the mandibles, maxillæ and labium respectively. The position of these appendages has already been noted and little need be said about their associated mesoderm beyond the fact that it develops in the same way as in other regions of the body behind (Eastham, 1927) and that each somite lies against the base of its appropriate appendage (fig. 50). The head mesoderm in front of the mandibles, however, develops from the proliferated mass of cells hitherto designated anterior mesenteron rudiment. The area of such proliferation marks the position of the future mouth and the cells arising on the yolk side of the germ band at this place are continuous with the mandibular mesoderm behind them.

Three distinct mesodermal units are formed: an anterior mass of head mesoderm is separated from the anterior side of the mesenteron rudiments; this is the labral mesoderm belonging to the protocerebral segment of the head (fig. 55). It passes forwards and part of it enters each of the hollow labral lobes. A more posterior part of it separates off and lies behind the epipharyngeal lobes overhanging the mouth (figs. 53 and 54). Cavities of doubtful significance may appear in both these mesoderm masses (figs. 54 and 55), but the cavities are so irregular and so unlike those of the well-defined somites of the body that it is doubtful whether they correspond to the latter. The cells surrounding the cavities are not arranged in regular radial fashion (compare figs. 55 and 50); and I am of the opinion that the looseness of organisation of the preoral mesoderm accounts for the appearance of spaces between the cells—such spaces not being homologous with those of true somites.

It is important to notice that the mesoderm which lies behind the epipharyngeal lobes results from a secondary separation of that mesoderm, the bulk of which passes to the labral lobes. For this reason I assume that only one segment is involved; though examination of the appendage-like structures which develop there might lead one to assume the presence of two preoral segments. In support of this view only one neuromere is found in this region.

The second paired mesodermal mass to be separated from the anterior mesenteron rudiment is the antennal. This spreads out at first as a thin layer (fig. 52) in a postero-lateral direction and by increase in size forms an appreciable rounded mass of cells at the base of each antennal lobe. Here again no somite cavity is formed similar to that, say, in the mandibular somite (fig. 50). More posteriorly still, two smaller groups of mesodermal cells are found behind the antennal mesoderm. These lie against the premandibular appendages, and the mass of one side is united to its fellow of the other by a prominent strand of mesodermal cells, occupying a postoral position exactly corresponding to that which is preoral, and in association with the epipharyngeal lobes mentioned above. This postoral mesoderm belongs to the premandibular or intercalary segment. The final condition is one in which the mesenteron rudiment lies encircled by the mesoderm of the three anterior head segments. The anterior head mesoderm is connected across the front of the mouth by cells lying behind the preoral or labral lobes. It is continuous on either side with antennal and premandibular mesoderm. The latter in turn is continuous with the mesoderm of the mandibles. Text-fig. 1 represents a diagrammatic reconstruction showing the relation between segments, appendages and mesoderm.

The origin of the mesoderm of the cephalic segments in front of the mandible has hitherto been obscure in all but the more generalised insects. STRINDBERG (1913) correctly describes the condition in *Bombyx*, but does not give an adequate account of the mode of development. NELSON (1915) briefly describes head mesoderm applied to the surface of the stomodæum, behind which lie two lateral groups of mesoderm in the antennal region. The origin of this, whether from anterior mesenteron rudiment or no, is not clear. The most complete account is that given by WIESMANN (1926) on the embryology of *Carausius*, where complete somites are formed in all segments. Since no anterior mesenteron rudiment is present in this insect the mode of origin of the anterior cephalic mesoderm must be quite different from that of the same structure in *Pieris*. This author states that all head and body mesoderm arises in a uniform manner. WHEELER (1889) in *Doryphora* finds that the mass of lower layer cells in the head arises in a similar manner to that of the mesoderm behind. It only differs from such mesoderm in that it is a cell mass destined to give rise to mesoderm and ectoderm. This is a condition similar to that of *Pieris*, except for the fact that in the latter the anterior mesenteron rudiment arises by proliferation. There is on the whole a lack of detailed information on this



TEXT-FIG. 1.—Diagrammatic representation of head segments showing appendages and associated mesoderm. For lettering see p. 47.

subject, neuromeres and appendage and segment formation being commonly described, but mesoderm both in the matter of its development and its relation to appendages being incompletely investigated.

It is not surprising to find that the mesoderm of the oral region arises differently from that behind. It must quite obviously be referred to the association, which is a secondary one, which exists between the mouth and segments behind it. The original anterior position of the mouth cannot be doubted. In this original position there would be a clear distinction perhaps between the anterior endoderm rudiment and the cephalic somites behind it. There would, therefore, be no reason for the most anterior of these to differ in mode of development from those of the body. Such a condition exists still in the Orthoptera, where definite somites with large cavities occur in the head segments and where these arise uniformly with those of the thorax and abdomen (*vide* WIESMANN (1926) and all other works on Orthopteron development quoted by him). The shifting back of the mouth which has occurred in all Arthropods in varying degree, ultimately bringing the antennal and intercalary segments into preoral and mandibular segment into paroral positions, would naturally tend towards a closer association between the mesoderm of those affected segments and the enteron rudiment arising at the mouth. Though the mouth continues to arise in front of the antennal segment in all insects its future more posterior position is foreshadowed by the crowding round it of antennal and premandibular somites. Thus in *Pieris* the antennal mesoderm arises only slightly posteriorly from the anterior mesenteron rudiments ; the premandibular or intercalary mesoderm arises posterolaterally from the same structure.

At one time these must have arisen in the same way as the mesoderm behind, as in Orthoptera. The present posterior origin of the mouth and anterior mesenteron rudiment pushes the antennal and intercalary mesoderm into more lateral positions, and this prevents them from participating in the same method of development experienced by the uninterfered-with segments behind them. In this way can be explained the anterior masses of cells called mesentoderm, *i.e.*, common masses of cells in the head, from which endoderm and mesoderm arise, as in Coleoptera (WHEELER, 1889 ; HIRSCHLER, 1909) in Lepidoptera, and most probably Hymenoptera (*Apis*, NELSON, 1915).

The mouth and its associated endoderm pass back earlier and earlier in ontogeny and a fusion with certain cephalic somites results. If such is the case we can easily understand why such head segments fail to develop clearly in the most specialised insects. Only those somites which are destined to form a tubular or sac-like organ would possess a cavity, the others would fail to develop a cavity within them. Thus in *Apis* (NELSON, 1915) the only distinguishable head somite is the antennal which forms the cephalic aorta. In *Donacia* (HIRSCHLER, 1909) only premandibular and labial cœlom develops and here it is the premandibular cœlom which forms the aorta. The retention of the labial cœlom is to be explained as being due to its more posterior position ; it, therefore, suffers least from interference from the oral cell mass. In *Pieris* the antennal cœlom develops late, the premandibular being reduced. Mandibular, maxillary and labial cœloms being distinctly postoral in development are not involved with the mouth and develop normally (fig. 50).

It might be pointed out here that in a hæmocœlic animal, like an insect, the retention of cœlomic pouches can only have a phylogenetic significance. This is indicated by their clear retention in generalised forms, such as the Orthoptera, and their absence in Muscid Diptera. Any process of development upsetting their normal method of growth will cause their obliteration, except where a cavity is destined to be formed by them in the adult, *e.g.*, antennal cœlom, which forms the aorta in *Apis*, and premandibular cœlom, which forms the aorta in *Donacia*.

(*c*) *The Labrum.*—The question as to whether the labral lobes are appendages belonging to the protocerebral segment of the head has been dealt with by HEYMONS (1895). This author, and indeed most others who have written since then, objected to their inclusion in the appendage category, on the grounds that they lie median to their neuromeres, viz., the protocerebral lobes of the brain, whereas true appendages lie lateral to their neuromeres. Such grounds for their occlusion are hardly reasonable, since the mesoderm masses—even definite somites in *Carausius*—lie behind them and pass into their cavities. If mere position with regard to neuromere is to preclude their designation as appendages the same must apply to their mesoderm, which should by the same rule be placed in some other category. Yet no one seems to deny that the mesoderm represents a pair of mesoblastic somites. We have in this part of the embryo a pair of hollow ectodermal lobes, associated with mesoderm and with a neuromere, *i.e.*, all the necessary criteria indicative of a segment. TOYAMA (1902) in *Bombyx*, GRABER (1890) in *Pieris*, WIESMANN (1926) in *Carausius*, figure two labral lobes, the latter author describing the development of the mesodermal somites behind them.

The nerve ganglia of the labral segment are certainly lateral to them instead of occupying a median position. This must be due to the requirements of brain growth, the neuromeres here forming the major portion of the brain. Head appendages during development, or subsequently, are associated with the mouth. If the protocerebral appendages, somites and neuromere, were to retain the spatial relations which obtain in the body region, brain growth of the first segment would push its segmental appendages to a position at the sides having no reference to the mouth. A reversal of position between the appendage and somite on the one hand and the neuromere on the other has enabled these appendages to retain their functional connection with the mouth. I suggest, therefore, that all the elements necessary to form a complete segment are present in the first segment of the head in *Pieris*, a pair of somites, a neuromere and a pair of limbs.

With regard to the epipharynx the facts that it arises as a paired structure, quite as distinct as the premandibular appendages, and that it possesses a small double mass of mesoderm which passes into it as it develops into the median lobe, may tempt one to the conclusion that another segment is concerned here. It must be remembered, however, that the mesoderm of the epipharynx is secondarily separated from the protocerebral mesoderm. This together with the fact that no neuromere is present, leads one naturally to the conclusion that there is no segmental significance to be attached to the at first bifid epipharynx. I contend therefore that the embryology of *Pieris* fully substantiates the orthodox conception of the six-segmented constitution of the insect head.

Head segmentation is notoriously a subject beset with many pitfalls to the embryologist, especially in studies of the more specialised groups of insects. In the latter forms somites tend to become reduced, or even obliterated, and any criticism of Orthopteron investigations, based on the development of a specialised insect like *Pieris*, must always be of a guarded nature. WIESMANN (1926) in a clear review of the subject of insect head segmentation points out the great advance which has been made possible by embryological study. The earliest researchers, working by purely anatomical methods, concluded that two, three or four segments were involved; thus PACKARD (1898) found two, MACLEAY (1863) found four segments. Even the earliest embryological work, however, did not clear the situation and we still find differences of opinion, such as those of PACKARD (1871) and GRABER (1888) who found four segments, while ZADDACH (1854) found six, recognising the eye-bearing region as a distinct segment.

VIALLANES (1887) and (1891) made a great step forwards by investigating brain constitution, arriving at the conclusion that there were three neuromeres in the supra-œsophageal nerve mass. Though PATTEN (1884) and WHEELER (1889) and CARRIÈRE and BÜRGER (1898) at first disagreed with VIALLANES, and found four segments in the brain region, they later with HEYMONS (1895) accepted the six-segmented conception, three segments for the brain and three for the subœsophageal mass.

JANET (1899, *b*) again, by morphological study of *Formica*, found three segments indicated by the visceral ganglia, three by the brain, and three by the subœsophageal mass. VERHOEFF (1905), basing his conclusions on an examination of the tentorium and muscles, and BERLESE (1909) decided on eight segments, three segments—a labral, a clypeo-pharyngeal and a fronto-ocellar—being indicated in front of the antennæ. All agreed as to the mandibular, maxillary, and labial segments, but FOLSOM (1900) found in *Anurida maritima* a segment between the mandibles and maxillæ. This is now discredited, having been contradicted by PHILIPTSCHENKO (1912). In 1901 HEYMONS found a preantennal segment in *Scolopendra*, and on this modified his views on insect head segmentation, and it is with HEYMON'S later view that WIESMANN'S work (1926) finds closest agreement. STRINDBERG (1913) following HOLMGREN (1908) in his anatomical investigations on Termites finds a six-segmented head, but prefers to regard the antennal segment as preoral, stating that the antennal segment curves forward in front of the mouth. If this is so it indicates an embryonic condition more nearly approaching the adult state than has hitherto been found in an insect. The work of LEUZINGER, WIESMANN and LEHMANN (1926) on *Carausius* is of especial interest. They find that the labrum, arising as two ectodermal lobes, has two distinct mesodermal somites associated with it. Between the labral and antennal cœlom a further pair of mesodermal pouches is found, lying near the anterior third of the stomodœum. These are pre-antennal and because of their somites and a pair of associated limb-like outgrowths are said to represent a distinct segment. No neuromere, however, is associated with this segment. These workers, therefore, conceive of the insect head as consisting of seven segments, and in this they agree with HEYMONS (1901).

This matter has already been commented upon by MANTON (1928), who points out that no somite has been found between the acron and precheliceral somites in Arachnids, nor between pre-antennulary and antennulary somites of Crustacea, nor between labral and antennary somites in any other insect. MANTON prefers to regard the preantennary (the additional somite of *Carausius*) as the lower half of the somite in front of it, viz., labral. This conclusion is supported by the development of a secondary mesoderm mass from the preoral mesoderm in *Pieris*. This, it will be remembered, lies within the embryonic epipharynx, overhanging the mouth, and the short-lived existence of two distinct mesoderm units, one in front of the other, but both in front of the mouth, may be a purely secondary condition due to the splitting of the mesoderm of one segment so as to appear as if belonging to two.

In justice to the excellent work of WIESMANN and his colleagues (1926), it must, however, be pointed out that at present there is no evidence that their preantennary somites are not real. If they are, then the epipharyngeal lobes and associated mesoderm of *Pieris* will have to be regarded as vestiges of a head segment, which up to the present time has been observed only in the Orthopteron *Carausius*.

DENIS (1927) dealing with the matter of head segmentation, divides the head into the Acron (Trochophorium) and a metameric part or Arthropodium. On evidence of an anatomical nature, which is not clear, he maintains that behind the acron is a pre-antennary segment whose neuromere is fused with the Protocerebrum to form a Syncerebrum; its somite is fused with that of the antenna and its appendages have disappeared. A special muscle—the oculo-motor of *Anurida*—appears to be the main evidence for an extra segment here. Then follows the antennary segment (antennulary of Crustacea), behind which is the premandibular (antennary of Crustacea). The superlingual is the next segment, whose neuromere fuses with the subœsophageal nerve mass and whose musculature belongs to the ventral side of the stomodæum. Its cœlom is fused with that of the preceding segment and it possesses a separate tentorial apparatus. These segments, with those of mandibles, maxillæ and labrum give a head consisting of acron and seven succeeding segments. This conception cuts across all recognised views on head segmentation to such an extent as to render discussion difficult. It is a risky thing to define a segment by the presence of a muscle or a tentorial invagination alone, and from the congested account given by this author one is driven to the conclusion that his ideas rest too much on anatomical studies and too little on embryological evidence.

The older work of CARRIÈRE and BÜRGER (1898) must be further mentioned. These workers found a development of labral lobes in *Chalicodoma* very similar to that described here for *Pieris*. They also found small solid appendicular structures in front of the antennæ, which they called the appendages of the "Gehirnsegment." These have no counterpart in *Pieris*, and indeed have been found in no other insect. That they are appendages is very doubtful, since they are solid and no mesoderm is associated with them. A single fold develops over the mouth invagination, called by them the "Vorderdarmfalte." This obviously corresponds to what I have called epipharynx in *Pieris*. It is a single structure at all times, and lateral to and in front of it develop the labral lobes. These authors' figures show a fusion between it and the labral lobes, though they do not mention the fact. In *Chalicodoma* it is obviously non-appendicular, and if homologous with the epipharynx in *Pieris* affords further evidence for my view that this does not indicate a further head segment.

Though the evidence in *Pieris* is in favour of regarding the labral lobes as appendages of the first head segment it must be borne in mind that the labrum has not a bifid origin in all insects. In most Orthoptera and Dermoptera it is unpaired, as for instance in *Blatta* and *Ziphidium*, WHEELER (1889 and 1893); in *Mantis*, VIALLANES (1891), and in *Forficula*, HEYMONS (1895). In *Carausius* it is paired, WIESMANN (1926). Among Hymenoptera it has an unpaired origin in *Apis*, NELSON (1915), but a paired origin in *Chalicodoma*, CARRIÈRE and BÜRGER (1898). According to KOWALEVSKY (1871), HEIDER (1889), GRABER (1890), PATTEN (1884), and NUSBAUM (1888), it has a paired origin in Coleoptera, while GRABER (1890), TOYAMA (1902), TICHOMIROFF (1882) find the labrum to develop from two lobes in Lepidoptera. There is no evidence as to which is the primitive condition, but since the labrum is an unpaired organ in the adult, it is difficult to understand why it should develop from two lobes in any insect, if that double form has other than a phylogenetic significance.

The table on p. 36, taken from WIESMANN (1926), indicates the main views on the subject.

(*d*) *Internal Skeleton*.—Soon after the appendages have developed on the head, a series of deep invaginations arises between them. These are tubular ingrowths of ectoderm, and it is for the most part from them that develop the chitinous beams which support the cranium and give attachment to important muscles of the mouth parts.

In *Pieris* their development is at first simple. Behind the antennæ, mandibles, maxillæ and labium they arise in perfect metameric fashion (fig. 56, Plate 9). Of these, the pair arising behind the antennæ form the anterior arms of the tentorium. The pair behind the mandibles give rise to the mandibular apodeme. The posterior arms of the tentorium arise behind the maxillæ, while the invaginations behind the labium develop into the silk glands. A pair of small invaginations also arises immediately behind those of the anterior arms of the tentorium.

Taking each of these structures in turn, we find that the first pair, or antennal, pass backwards and downwards along each side of the brain. On reaching the level of the floor of the œsophagus, behind the circumœsophageal commissure, each meets the posterior tentorial invagination, from which point an inwardly directed process passes

WIESMANN, 1926, HEYMONS, 1895, GOODRICH, 1898, HEIDER, 1889.	VIALLANES, 1891, CHOLODKOVSKY, 1890, PACKARD, 1898.	HOLMGREN, 1908.	HEYMONS, 1901.	PATTEN, 1884.	WHEELER, 1889, CARRIÈRE, 1898.	WIESMANN, 1926.	FOLSOM, 1900.	VERHOEFF, 1905.	JANET, 1899.
Number of segments.									
6	6	6	6 (7)	7	7	7 (or 6+1)	7	8	9
Primary head segment			Acron			Acron Rudimentary	Oral	Labral	Prostomod. Deutostomod.
				Brain Seg.	Labral	Labral Segment = Acron + Labrum		Clypeo pharyng.	Tritostomod. Protocer.
	Ocellar segment	Optic Seg.	Preant.	Labral	Brain Segment	Preant.		Fronto-ocellar	
Ant.	Ant.	Ant.	Ant.	—	—	—	—	—	Ant.
Intercal.	Inter.	Labral	Int.	—	—	—	—	—	Inter.
Md.	Mand.	Mand.	Md.	—	—	—	—	—	Md.
Mx.	Mx.	Mx.	Mx.	—	—	—	—	—	Max.
Lab.	Lab.	Lab.	Lab.	—	—	—	—	—	Lab.

under the œsophagus to join its fellow, giving support to the latter and forming a protective plate above the subœsophageal ganglion (fig. 57).

The next pair of invaginations arise at nearly the same point as the latter. Actually they pass inwards immediately behind the anterior arms of the tentorium and pass backwards and downwards on the outerside of the tentorial structures, curving outwards as they do so. They terminate against the body wall in the mandibular segment, joining at the same time an outward process of the mandibular apodeme, next to be considered. These short invaginations, which I regard as belonging to the embryonic premandibular segment, form the tendon of the extensor mandibular muscle.

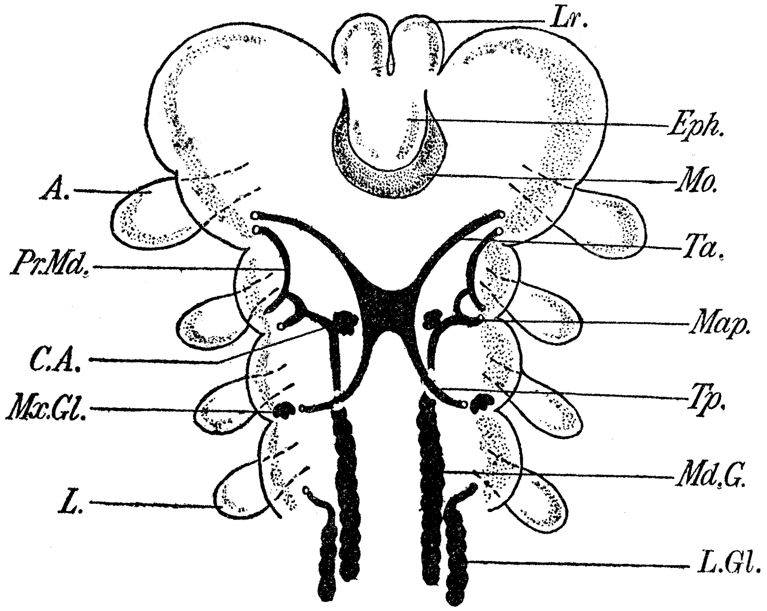
From behind the mandible a strong tubular ingrowth is found lined prominently with chitin. This passes backward along the side of the stomodæum. In transverse section it is crescentic and a branch from it passes outward to meet the premandibular apodeme or extensor mandibular tendon. The main part of the mandibular invagination proceeds on its backward course, and at a point just behind the brain ends in a long tubular gland (fig. 46).

The course of the gland during development is of interest. It passes straight back parallel to the œsophagus, turns abruptly upwards at the level of the anterior border of the yolk stalk and terminates in the newly-formed hypodermis at this point. As the embryo gets more pinched off from the yolk, the yolk stalk becomes narrower and the gland's connection with the hypodermis is carried thereby further back and the gland in consequence lengthened. So long as there is even the slightest open connection between the dorsal side of the embryo and the yolk, one finds the mandibular gland connected to the dorsal body wall ; fig. 39 shows this gland on its upward course towards the hypodermis in this position. The main part of the mandibular invagination forms the tendon of the flexor mandibular muscle. When the head of the caterpillar is recognisable in the egg a band of cells connects the proximal parts of the mandibular apodeme of the two sides, forming a connecting strut which lies immediately behind the front wall of the head capsule.

The maxillary invaginations arise between the first and second maxillæ (figs. 56 and 57). They pass forward into the cavity of the head and form the posterior arms of the tentorium. Joining the anterior tentorial arms at the point where the body of the tentorium passes under the œsophagus to join the tentorial arms of the other side, it will be seen that the whole tentorial structure which develops is H-shaped, the cross-bar being the tentorium proper, and the legs formed by anterior and posterior arms respectively.

The corresponding invaginations which arise against the posterior inner sides of the bases of the second maxillæ form the silk glands. In the course of development the two second maxillæ fuse in the middle line, and in so doing the openings of the silk glands are brought together (fig. 60). In this way the larval condition is attained, where only one external opening is found.

A further gland must be mentioned. It is difficult to decide to which segment it belongs. TOYAMA (1902) calls it the outer labial or hypostigmatic gland. It arises in *Pieris* in such close association with the posterior arms of the tentorium that I prefer to regard it as maxillary rather than labial (fig. 59). On this view we should have a series of head glands belonging to the mandibular, maxillary and labial segments. The gland arises as an invagination (fig. 59) but quickly loses any sign of its tubular origin, passes back alongside the mesenteron rudiment and terminates just behind and below the prothoracic spiracles. A similar gland was found by TOYAMA (1902) in *Bombyx mori*, and on account of its ultimate spiracular association was called by him the hypostigmatic gland, of unknown function. Text-fig. 2 shows in diagrammatic manner the relation between the internal skeleton of the head and the glands to their segments.



TEXT-FIG. 2.—Diagrammatic representation of head segments after absorption of the premandibular segments, showing ectodermal invaginations to form the tentorium and mandibular apodeme, and the associated glands. (For lettering see p. 47.)

The development of the internal skeleton of the head and the associated glands in *Pieris* agrees in the main with the findings of TOYAMA (1902) on *Bombyx mori*. I differ from him, however, in the segments of the head to which the various structures belong; to this I shall refer later. There seems to be no other reference to the hypostigmatic gland of TOYAMA, unless this is homologous with the cephalic spiracle described by NELSON (1915) in *Apis*. In *Apis* NELSON describes a spiracular invagination which arises in front of the second maxillæ, and from it arise the principal cephalic tracheæ. The spiracle closes and its tracheæ become connected to the main longitudinal trunk in the thorax. Other workers on tentorial development are HEIDER (1889), HEYMONS (1895), CARRIÈRE and BÜRGER (1898) and RILEY (1904), and there is substantial agreement between the accounts given by these authors.

Tentorial invaginations and silk glands have often been homologised with tracheal invaginations of the post-cephalic segments (*vide* PALMEN, 1877, HATSCHEK, 1877, WHEELER, 1889, and CARRIÈRE and BÜRGER, 1898), but the importance of these structures as affording confirmatory evidence on which to found a conception of head segmentation seems to have been much neglected. Though there is general agreement between the findings of the investigators quoted, in the number and fate of cephalic invaginations, there is considerable difference of opinion as to the segments to which the several invaginations belong. Thus RILEY (1904), working at *Blatta*, says the anterior arms of the tentorium arise behind the antennæ on the mandibular segment. HEYMONS (1895), in *Forficula*, however, says they belong to the antennal segment. TOYAMA (1902) states that they belong to the mandibular segment in *Bombyx mori*; while according to HEIDER (1889) they arise in the mandibular segment in *Hydrophilus*. All agree in that they arise in some position intermediate between the antennæ and mandibles. As for the posterior arms, RILEY (1904) ascribes them in *Blatta* to the labial segment, TOYAMA (1902) to the maxillary segment. But all agree in the origin of the mandibular apodeme from the mandibular segment.

If one examines all these accounts one is bound to accept the conclusions of NELSON for *Apis* (1915), viz., that the three main pairs of invaginations arise behind the antennæ, mandibles and maxillæ respectively, the silk glands, forming the fourth, arising behind the labium. They form a distinct series, and though intersegmental in position, give a clear indication of the segmentation of the head in that region. Such a segmental interpretation is, however, impossible on TOYAMA'S or RILEY'S interpretation, for they attach the anterior arms of the tentorium as well as the mandibular apodeme to the mandibular segment; and RILEY by connecting the posterior arms of the tentorium to the labium, again renders any segmental interpretation of the tentorium impossible, by omitting the first maxilla from any connection with tentorium.

From my study of the development of the tentorium in *Pieris*, I have come to the conclusion that an anatomical study of the tentorium alone is not sufficient to enable one to interpret it properly. During the course of development the shape of the head changes considerably. From their originally evenly spaced positions on the cephalic part of the germ band, the whole of the mouth parts and antennæ become congregated in the region of the labium. In this process the preoral region, consisting of labrum, clypeus and frons, becomes greatly enlarged and the intersegmental regions behind this greatly reduced. While this big upheaval is going on, tentorial invaginations change their positions considerably. For instance, the posterior arms of the tentorium, which undoubtedly arise immediately behind the maxillæ, as fig. 56 clearly shows, ultimately find themselves in a position posterior to the labium (fig. 57). It must surely be movements of this kind, which account for the difference of opinion related above as to the segmental or intersegmental positions of the tentorial invaginations.

The cephalic spiracle, discovered by NELSON in *Apis* in front of the second maxilla, must be considered here. He describes it as belonging to the second maxilla. I have already pointed out its close similarity, on account of position and the fact that it loses its hypodermal connection, to the hypostigmatic gland in both *Bombyx* and *Pieris*. The above-mentioned embryologists who homologised tentorial and silk gland invaginations with spiracles contended that the anterior tentorial arms represented an original first spiracle and the posterior tentorial arms of the second maxillary segment corresponded to the third pair of spiracles. The additional pair of spiracles on this same segment in *Apis* precludes NELSON from maintaining the homology suggested by PALMEN (1877), HATSCHEK (1877), and CARRIÈRE and BÜRGER (1898), viz., between tentoria and spiracles. This, however, does not prevent us from employing the cephalic invaginations in a determination of head segmentation.

The following is my interpretation of the cephalic invaginations. In view of the fact that all investigators are in agreement as to the origin of the main invaginations from behind the antennæ, mandibles, first and second maxillæ, I maintain that we have here another criterion for testing our conception of the segmental constitution of the head. It is of the greatest significance to notice that a second invagination arises immediately behind that of the anterior tentorial invagination. This can reasonably be associated with the very reduced premandibular segment, though by the time this pair of invaginations is easily recognisable the segment itself has been absorbed. We have, therefore, the anterior arms of the tentorium associated with the antennæ, the short invaginations of the flexor mandibular tendon belong to the premandibular segment, the mandibular apodeme to the mandible, the posterior tentorial arms to the maxilla and the silk glands with the labium. One is tempted in making these assertions to recall the analogous vertebrate condition and homologise the mouth and labrum with these several apertures and their respective appendages, just as the vertebrate mouth has been regarded as the homologue of the gill clefts.

It might be objected that it requires a very speculative mind to homologise a tentorial invagination, which is purely skeletal, with the purely glandular structures—the silk glands of the labium. The difficulty, however, diminishes considerably when we remember that a gland is associated with the mandibular apodeme and that a gland arises close beside the origin of the posterior arms of the tentorium which I maintain are maxillary. It may be that, associated with each segment of the head, there was originally a gland. RILEY (1904) suggests that these might be homologous with dermal glands of Annelids. As the head became more and more elaborated into a box to protect the brain and bear masticatory organs, the need for internal support arose. This support was afforded by segmental invaginations which arose against these glands. It is not surprising that the extent to which these structures are developed differs in the various regions of the head. The mandible being most powerful in mastication possesses not only the strongest apodeme belonging to its own segment but has brought into its service the smaller apodeme belonging to the evanescent segment in front of it. The labium, being for the most part a mere carrier of the silk gland aperture (in *Pieris*) or a more or less passive organ bounding the posterior side of the mouth, is provided with no special apodemes but the gland is well developed. Antennary and maxillary invaginations are well developed and devoted to forming the tentorium.

Were it not for the fact that NELSON (1915) has described a cephalic spiracle in *Apis*, one would like to adopt the tentorium-spiracle homology and point out the common association of glands with spiracles in the main body region. SNODGRASS (1928), in an extensive treatise on the morphology of the insect head, points out that the anterior tentorial arms arise in front of the mandibles, while the posterior arms originate from depressions in the post-occipital suture. This is the adult condition, but if the post-occipital suture is, as he supposes, an intersegmental boundary between maxillary and labial segments, his description coincides in a remarkable degree with that of the development given above.

Plan of Head Segmentation.

Segment.	Somite.	Appendage.	Neuromere.	Tentorial organ.	Gland.
1	Present	Labrum	Protocerebrum	—	—
2	Present	Antennæ	Deutocerebrum	Tent. Ant.	—
3	Present	Premandibles	Tritocerebrum	Extensor Mand.	—
4	Present	Mandible	Sub-œsoph. 1	Flexor Mand.	Mand. gland
5	Present	Maxilla	Sub-œsoph. 2	Tent. post.	Max. gland
6	Present	Labium	Sub-œsoph. 3	—	Silk gland

15. *Summary.*

(1) Development of body from the gastrulation stage to the complete caterpillar is described. Blastokinesis consists of a straightening out of a dorsally flexed embryo, followed by a prominent ventral flexure, which finally brings the last abdominal segment into close approximation to the head.

(2) Appendage development is described. The antennæ are postoral, but early assume their ultimate preoral position. Premandibular appendages occur, but quickly disappear. A pair of post labial lobes, of no segmental significance, develop from the anterior sides of the basal joints of the prothoracic appendages; these persist in the fully formed caterpillar. Paired appendages occur on all abdominal segments, those on the third to sixth and the tenth remaining as prolegs. The first abdominal appendages known as pleuropodia develop into glandular structures whose maximum function coincides with the period of later blastokinesis and the release of the embryo from the embryonic membranes. The significance of these is discussed.

(3) Mesoderm consists of small lateral segmental hollow somites and an irregular mass of cells below connecting the mesoderm of the two sides. The actual somite gives rise to cardioblasts, visceral muscles, lateral body-wall muscles and fat body. The sub-somitic mesoderm develops into the ventral and ventrolateral body musculature, and

into the intrinsic and extrinsic muscles of the legs in leg-bearing segments. Median mesoderm connecting the mesoderm of the two sides forms ventral transverse muscles, but is largely dissipated in the formation of blood and other cells which disintegrate in the yolk.

(4) Segregation of neuroblasts from dermatoblasts along two parallel tracks one on each side of the middle line leads to the formation of two lateral nerve cords. A median cord is similarly developed from cells in the roof of the neural groove. From the lateral cords, the segmental ganglia and their longitudinal commissures develop. Segmentally the median cord gives rise to transverse commissures.

Brain development occurs in a similar manner to that of the nerves forming the ventral cord. Of the three first cephalic segments the first is largest and contributes most to brain development. The tritocerebral lobe develops from ectoderm in the region postero-lateral to the mouth, and immediately after this the segment disappears. A supraœsophageal commissure develops from the ectoderm to join the two protocerebral lobes together and from the inner sides of the tritocerebral lobes there develops a subœsophageal commissure.

The stomatogastric system takes its origin in the dorsal wall of the stomodæum.

(5) Of the alimentary canal, fore and hind-gut develop as ectodermal invaginations, the malpighian tubules arising as three pairs of diverticula of the proctodæum. The mid-gut develops from two masses of proliferated cells lying first in the regions of mouth and anus; they are carried inwards as the stomodæum and proctodæum develop. Cells from these two masses pass over the mesoblastic somites in two strings, which by growth on their median side form a complete sheet supporting the yolk. The endoderm question is discussed and an attempt is made to reconcile opposing views with one another.

(6) The heart develops from cardioblasts which are differentiated from the dorsal wall of the somites. Cardioblasts of the two sides approach one another in the mid-dorsal line as the body-wall development is completed and in this way a tubular heart is formed.

(7) The cephalic aorta develops as a tubular cavity in the conjoined masses of antennal mesoderm above the stomodæum. It remains open in front and joins up with the heart in the region of the first thoracic segment.

(8) There are at first 10 spiracles, 2 thoracic and 8 abdominal. Of these, the metathoracic spiracle closes, leaving only one prothoracic and 8 abdominal functional spiracles in the caterpillar.

(9) Blood cells arise from median mesoderm, but similar cells are released throughout early stages of development from any part of the mesoderm. These appear to have a digestive action on the yolk. Such cells also take their origin in the anterior endoderm rudiment.

(10) Groups of large cells develop in the ectoderm behind each abdominal spiracle. These pass inwards to persist as œnocytes, which retain their post-spiracular association.

(11) The further development of the amnion is described up to the time when it

becomes severed from the embryo in the prothoracic segment. In this severance a certain amount of yolk is cut off from the embryo and remains between the amnion and serosa, to be eaten by the newly hatched caterpillar.

(12) The corpora allata arise as paired masses of cells from the same invagination which produces the mandibular apodeme in the head.

(13) Head development is described. The labrum arises as a pair of lobes on the protocerebral segment; these fuse at their bases, pass backwards with the mouth and become connected to the epipharynx, which is slightly bifid at its first appearance.

Preoral mesoderm becomes secondarily divided into an anterior part, which passes into the hollow labral lobes, and a posterior part, which lies against the inner side of the epipharynx.

The segmental significance of this region is discussed and the suggestion is put forward that the labral lobes are true appendages of the first head segment.

Antennæ, mandibles, maxillæ and labium have a typical development. Antennary mesoderm forms the cephalic aorta; mandibular, maxillary and labial mesoderm forms the muscles of these organs and arises in a manner similar to that of the body region behind. Premandibular mesoderm becomes converted into a mass of vacuolated cells underlying the junction of fore- and mid-gut and known as the subœsophageal body.

Paired ectodermal invaginations in the head give rise to the tentorium, to chitinous attachments for the mandibular muscles, and to glands in the mandibular, maxillary and labial segments. The antennary invaginations form the anterior arms of the tentorium. Premandibular invaginations form the tendons of the extensor mandibular muscles. Mandibular invaginations give rise to the mandibular apodeme, a further invagination from this being prolonged backwards into the body as the mandibular gland. The posterior arms of the tentorium arise as ectodermal invaginations in the maxillary segment. These join the anterior tentorial arms at the body of the tentorium which passes under the œsophagus. Behind the posterior tentorial invaginations a further ectodermal intucking develops into a hypostigmatic gland, which loses its connection with the hypodermis.

Silk glands develop as invaginations on the postero-inner sides of the second maxillæ.

The above ectodermal invaginations, forming chitinous and glandular structures, give further support to the orthodox conception of the head's six segmental constitution.

LITERATURE LIST.

BAILLON, P. C. de, 1920. "Contribution anatomique et physiologique à l'étude de la Reproduction chez les Locustiens et les Grilliniens. (1) La ponte et l'éclosion chez les Locustiens." 'La Cellule,' vol. 31.

BERLESE, A., 1909. "Gli Insetti," vol. 1. 'Embriologia e Morfologia, Milano.'

BLUNCK, H., 1912. "Die Entwicklung der *Dytiscus marginalis* im Ei bis zur Imago," 'Z. Wiss. Zool.,' vol. 3.

CARRIÈRE, J., und BÜRGER, O., 1898. "Die Entwicklungsgeschichte der Mauerbiene (*Chalicodoma muraria* Fahr.) im Ei." 'Abhandl. K. Leop.-Carolin. Deut. Akad. Naturf.,' vol. 69.

CHOLODKOVSKY, N. A., 1890. "Zur Embryologie von *Blatta germanica*." 'Zool. Anz.' vol. 13.

DENIS, J. R., 1927. "Sur la constitution morphologiques de la tête des Insectes." 'Bull. Soc. Zool. France,' vol. 102.

EASTHAM, L., 1927. "A contribution to the embryology of *Pieris rapæ*." 'Quart. J. Micr. Sci.,' vol. 71, Part 3.

——, 1930. "The Formation of Germ Layers in Insects." 'Proceedings Camb. Phil. Soc., Biol. Reviews,' vol. 5, No. 1.

ESCHERICH, K., 1900. "Ueber die Bildung der Keimblätterbei den Musciden." 'Abh. K. Leopold-Carolin. Deut. Akad. Naturf.,' vol. 17.

——, 1901. "Das Insekten Entoderm." 'Biol. Centrabl.,' vol. 21.

——, 1902. "Ueber den sogenannten Mittelstrang der Insekten." 'Biol. Centrabl.,' vol. 22.

FOLSOM, E., 1900. "The development of the mouth-parts of *Anurida maritima*." 'Bull. Mus. Comp. Zool. Harvard College,' vol. 36.

FRIEDERICHS, K., 1906. "Untersuchungen über die Entstehung der Keimblätter, und Bildung des Mitteldarmes bei Käfern." 'Nova Acta, Abhandl. K. Leopold-Carolin. Deut. Akad. Naturf.,' vol. 85, No. 3.

GOODRICH, E., 1898. "On the relation of the Arthropod head to the Annelid Prostomium." 'Quart. J. Micr. Sci.,' vol. 40.

GRABER, V., 1888. "Ueber die primäre Segmentierung des Keimstreifens bei Insekten." 'Morph. Jahrb.,' vol. 14.

——, 1890. "Vergleichende Studien am Keimstreifen der Insekten." 'Denkschr. k. Akad. Wiss. Wien,' vol. 57.

——, 1891. "Beiträge zur vergleichenden Embryologie der Insekten." 'Denkschr. k. Akad. Wiss. Wien,' vol. 58.

HATSCHEK, B., 1877. "Beiträge zur Entwicklungsgeschichte der Lepidopteren." 'Jenaische Zeitschr.,' vol. 11.

HEIDER, K., 1885. "Ueber die Anlage der Keimblätter von *Hydrophilus piceus*." 'Abhandl. k. Akad. Wiss. zu Berlin,' 1885.

——, 1889. "Die Embryonalentwicklung von *Hydrophilus piceus* L." Teil I., Jena.

HEYMONS, R., 1894. "Uebersicht über die neueren Ergebnisse auf dem Gebiete der Insektenembryologie." 'Zool. Zentrbl.,' Jahrg. I.

——, 1895. "Die Embryonalentwicklung von Dermapteren und Orthopteren, unter besonderer Berücksichtigung der Keimblätterbildung." Jena.

——, 1897. "Die Zusammensetzung des Insektenkopfes." 'Sitzungsbericht Ges. Nat. Freunde, Berlin.'

——, 1901. "Entwicklungsgeschichte des Scolopender." 'Zoologica,' vol. 33.

HIRSCHLER, J., 1909. "Die Embryonalentwicklung von *Donacia crassipes* L." 'Z. Wiss. Zool.,' vol. 92.

——, 1909, *a*. "On the development of the germ layers and the alimentary canal of *Gasteroidea viridula* Deg." 'Bull. Internat. Acad. Sci. Krakow,' 1909.

HOLMGREN, N., 1908. "Zur Morphologie des Insektenkopfes. I. Zum metameren Aufbau des Kopfes der *Chironomus* Larve." 'Z. Wiss. Zool.,' vol. 76.

HUSSEY, P. B., 1926. "Studies on the Pleuropodia of *Belostoma flumineum* and *Ranatra fusca*." 'Entomologica Americana,' vol. 7, No. 1.

IMMS, A. D., 1925. "A General Textbook of Entomology." (Methuen & Co., 1925.)

ITO, HIROWA, 1918. "On the Glandular Nature of the Corpora allata of the Lepidoptera." 'Bull. Imp. Tokyo Sericulture College,' vol. 1, p. 4.

JANET, Ch., 1899, *a*. "Sur les Nerfs céphaliques, les Corpora allata et le Tentorium de la fourmie." 'Mem. Soc. Zool. France,' tome 12.

——, 1899, *b*. "Essai sur la constitution morphologique de la tête de l'insecte." Paris.

KARAWAIEW, W., 1893. "On the embryonic development of *Pyrrhocoris apterus* (Russian)." 'Zapiski Kiev. Obsch.,' vol. 13.

KORSCHELT, E., 1912. "Zur Embryonalentwicklung des *Dytiscus marginalis* L." 'Spengels Zool. Jahr., Suppl. 15,' vol. 2.

KOWALEVSKY, A., 1871. "Embryologische Studien an Würmern und Arthropoden." 'Mem. l'Acad. Imp. Sci. St. Pétersburg,' tome 16.

LECAILLON, A., 1898. "Recherches sur le Développement embryonnaire de quelques Chrysomélides." 'Arch. Anat. micr.,' vol. 2.

LEUZINGER, H., WIESMANN, R., and LEHMANN, F. E., 1926. "Zur Kenntnis der Anatomie und Entwicklungsgeschichte der Stabheuschrecke *Carausius morosus* Br.," Gustav Fischer, Jena.

LONGCHAMPS, M. DE SELYS, 1904. "Recherches sur le Développement embryonnaire de l'Appendice du premier Segment Abdominal chez *Tenebrio molitor*." 'Bul. Classe Sciences Acad. Roy. Belg., Brussels,' vol. 4.

MACLEAY, W., 1863. 'New S. Wales Entom. Soc. Trans.', vol 1.

MANSOUR, K., 1927. "The Development of the larval and adult Mid-gut of *Calandra oryzæ* Linn., the Rice Weevil." 'Quart. J. Micr. Sci.,' vol. 71 (ii).

MANTON, S. M., 1928. "On the Embryology of a Mysid Crustacean, *Hemimysis lamornæ*." 'Phil. Trans.,' B, vol. 216.

NELSON, J. A., 1915. "The Embryology of the Honey Bee." 'Princeton University Press.'

NOACK, W., 1901. "Beiträge zur Entwicklungsgeschichte der Musciden." 'Z. Wiss. Zool.,' vol. 70.

NUSBAUM, J., 1888. "Die Entwicklung der Keimblätter bei *Meloe proscarabæus*, Marsh." 'Biol. Zentralbl.,' vol. 8.

NUSBAUM, J., and FULINSKI, B., 1906. "Ueber die Bildung der Mitteldarmanlage bei *Phyllodromia germanica* L." 'Zool. Anz.,' vol. 30.

—— ——, 1909. "Zur Entwicklungsgeschichte des Darmdrüsenblattes bei *Gryllotalpa vulgaris* Latr." 'Z. Wiss. Zool.,' vol. 93.

PACKARD, A., 1871. "Embryological studies on *Diplax*, *Perithemis*, and the Thysanurous genus Isotoma." 'Mem. Peabody Acad. Sci.,' vol. 2.

——, 1898. "A Textbook of Entomology," New York.

PALMEN, J. A., 1877. "Zur Morphologie des Tracheensystems," Leipzig.

PATTEN, W., 1884. "The Development of Phryganids, with a Preliminary Note on the Development of *Blatta germanica*." 'Quart. J. Micr. Sci.,' vol. 24.

PHILIPTSCHENKO, J., 1912. "Beiträge zur Kenntnis der Apterygoten. III. Die Embryonalentwicklung von *Isotoma cinerea* Nic." 'Z. Wiss. Zool.,' vol. 103.

RILEY, W. A., 1904. "The Embryological Development of the Skeleton of the Head of *Blatta*." 'Amer. Nat.,' vol. 38.

RIZZI, M., 1912. "Sullo sviluppo dell' novo di *Bombyx* (*Sericaria*) *mori* L." 'Redia,' vol. 8.

SCHWANGART, F., 1904. "Studien zur Entodermfrage bei Lepidopteren." 'Z. Wiss. Zool.,' vol. 77.

SCHWARTZE, E., 1899. "Zur Kenntnis der Darmentwicklung bei Lepidopteren." 'Z. Wiss. Zool.,' vol. 46.

SEIDEL, F., 1924. "Die embryonale Entwicklung der Geschlechtsorgane von *Pyrrhocoris apterus* L." 'Zeit. Morph. und Ökol. Tiere,' vol. 1.

SNODGRASS, R. E., 1925. "The Anatomy and Physiology of the Honey Bee." 'McGraw Hill Book Co., New York.'

——, 1928. "Morphology and Evolution of the Insect Head and its Appendages." 'Smithson. Miscell. Collect.,' vol. 81, No. 3.

STRINDBERG, H., 1913. "Embryologische Studien an Insekten." 'Z. Wiss. Zool.,' vol. 106.

TANQUARY, M. C., 1913. "Biological and embryological Studies on Formicidæ. III. Studies on the Embryology of *Camponotus herculeanus* and *Myrmica scabrinodis*." 'Bull. Ill. State Lab. Nat. Hist.,' vol. 9.

TICHOMIROFF, A., 1882. "Zur Entwicklungsgeschichte der Seidenspinners (*Bombyx mori*) im Ei." 'Arb. Labor. Zool. Mus. Moscau,' vol. 1.

TOYAMA, K., 1902. "Contributions to the Study of Silkworms. I. On the Embryology of the Silkworm." 'Bull. Col. Agric. Tokyo Imp. Univ.,' vol. 5.

TSCHUPROFF, H., 1903. "Ueber die Entwicklung der Keimblätter bei den Libellen." 'Zool. Anz.,' vol. 27.

VERHOEFF, K., 1905. "Ueber vergleichende Morphologie des Kopfes niederer Insekten, mit besonderer Berücksichtigung der Dermapteren und Thysanuren." 'Nova Act. Acad. Cæs. Leopold-Carol.,' vol. 84.

Viallanes, H., 1887. "Études histologiques et organologiques sur les Centres Nerveux et les Organes de Sens des Animaux Articulés." 'Ann. Sci. Nat. sér. 7, Zool.,' vol. 4.

——, 1891. "Sur quelques points de l'Histoire du Développement embryonnaire de la Mante religieuse (*Mantis religiosa*)." 'Ann. Sci. Nat. Zool.,' vol. 2.

Wheeler, W. M., 1889. "The Embryology of *Blatta germanica* and *Doryphora decemlineata*." 'J. Morph.,' vol. 3.

——, 1890. "On the appendages of the first abdominal segment of embryo insects." 'Trans. Wisconsin Acad. Sci.,' vol. 8.

——, 1893. "A contribution to insect embryology." 'J. Morph.,' vol. 8.

——, 1910. "Ants, their Structure, Development and Behaviour." 'New York, Columbia University Press.'

Wiesmann, R., 1926. See Leuzinger, H., Wiesmann, R., and Lehmann, F. E.

Zaddach, G., 1854. "Untersuchungen über die Entwicklung und den Bau der Gliedertiere." Heft 1. Berlin.

EXPLANATION OF PLATES 1–9.

Lettering.

A., Antenna; *Ab.*, Abdominal leg; *Am.*, Amnion; *A.Ms.*, Antennary Mesoderm; *Ao.*, Aorta; *Ap.*, Appendage; *As.*, Anus; *B.*, Blood cell; *C.A.*, Corpus allatum; *Cd.*, Cardioblasts; *Db.*, Dermatoblast; *Dc.*, Disintegrating cell; *Dg.*, Deutocerebral ganglion; *Dlm.*, Dorsolongitudinal muscle; *E.*, Ectoderm; *Elm.*, Elevator leg muscle; *E.M.*, Elevator mandibular muscle; *E.Ms.*, Epipharyngeal mesoderm; *En.*, Endoderm; *Ep.*, Epineural sinus; *Eph.*, Epipharynx; *Ex.M.*, Extensor mandibular muscle; *F.*, Fat body; *Gc.*, Germ cells; *Gd.*, Ganglion cells; *Gl.*, Gland cells; H., Hypodermis; *Hr.*, Heart rudiment; *Int.*, Intrinsic leg muscles; *Int.M.*, Intersegmental mesoderm; *L.*, Labium; *L.Gl.*, Labial gland; *Lr.*, Labrum; *Lr.Ms.*, Labral mesoderm; *M.*, Mandible; *Map.*, Mandibular apodeme; *Mc.*, Median cord; *Md.G.*, Mandibular gland; *Mg.*, Mid-gut; *Mm.*, Median mesoderm; *Mo.*, Mouth; *Ms.*, Mesoderm; *Mt.*, Malpighian tubule; *Mx.*, Maxilla; *Mx.gl.*, Maxillary gland; *Nb.*, Neuroblast; *Ng.*, Nerve ganglion; *N.Gr.*, Neural groove; *Nl.*, Neurilemma; *O.*, Œnocytes; *Op.*, Contact between Embryo and Yolk; *Os.*, Œsophagus; *P.*, Proctodæum; *Par.*, Parœsophageal commissure; *Pg.*, Protocerebral ganglion; *Pl.*, Post labral lobe; *Pm.*, Premandibular appendage; *P.Ms.*, Premandibular mesoderm; *Pr.Md.*, Premandibular apodeme; *Pr.Ms.*, Preoral mesoderm; *Pro.*, Protocerebral lobe; *S.*, Stomodæum; *Sb.*, Subœsophageal body; *Sc.*, Supraœsophageal commissure; *Seg.M.*, Segmental mesoderm; *Smu.*, Stomodæal muscles; *Som.*, Somitic mesoderm; *Sp.*, Spiracle; *Spg.*, Supraœsophageal ganglion; *Ssm.*, Subsomitic mesoderm; *Stg.*, Stomatogastric ganglion; *Stn.*, Stomatogastric nerve; *Sug.*, Subœsophageal ganglion; *T.*, Trachea; *Ta.*, Anterior tentorial arm; *Th.*, Thoracic leg; *Tp.*, Posterior tentorial arm; *Tri.*, Tritocerebral ganglion; *Vis.*, Visceral muscles; *Vis.C.*, Circular visceral muscles; *Vis.L.*, Longitudinal visceral muscles; *Vl.*, Ventro-lateral muscle; *Vlo.*, Ventro-longitudinal muscle; *Y.*, Yolk; *Ys.*, Yolk stalk.

Plate 1.

Figs. 1 to 6.—Views of whole embryos illustrating development of body form and the main features of blastokinesis. All are lateral, except fig. 1, which is ventral.

Fig. 7.—Transverse section through the third segment of the head, to show premandibular appendage of one side.

Fig. 8.—Lateral view of anterior region of newly-hatched caterpillar, showing post-cephalic spinous lobe.

Plate 2.

Fig. 9.—Transverse section of complete embryo, showing paired post-cephalic spinous lobes.

Fig. 10.—Transverse section of pleuropodium of an embryo of stage shown in fig. 6.

Fig. 11.—Transverse section through a segment of an embryo, showing arrangement of mesoderm after development of somites.

Fig. 12.—Longitudinal section through prothoracic region of embryo, to show early development of post-cephalic spinous lobe.

Fig. 13.—Longitudinal section through embryo after external segmentation, but before somite differentiation.

Fig. 14.—Longitudinal section of whole embryo in same stage as that of fig. 13.

Fig. 15.—Longitudinal section through three abdominal segments of an embryo after somite formation.

Plate 3.

Fig. 16.—Longitudinal section through median mesoderm in process of disintegration into blood cells, etc.

Fig. 17.—Transverse section (left half) through embryo, to show mesoderm in process of differentiation into visceral muscles, somatic muscles and fat body.

Fig. 18.—Longitudinal section through middle line of embryo after formation of blood cells and development of epineural sinus.

Fig. 19.—Transverse section (left half) through embryo, showing somite differentiation, muscle formation and development of nerve cord.

Fig. 20.—Transverse section (left half) through embryo in region of spiracle. Somite breaking down.

Fig. 21.—Transverse section (left half), showing cardioblast formation.

Fig. 22.—Transverse section (left half), to show late stage in visceral and somatic muscle formation, in second abdominal segment. Note germ cells, epineural sinus and big development of endoderm towards middle line.

Plate 4.

Fig. 23.—Transverse section, immediately after gastrulation before neuroblast formation.

Fig. 24.—Transverse section. Segregation of neuroblasts from dermatoblasts.

Fig. 25.—Longitudinal section through nerve ganglion, showing neurilemma derived from outer neuroblasts.

Fig. 26.—Transverse section through embryo in region of stomodæum. Protocerebral and deutocerebral neuromeres and segments defined. Antennary mesoderm dividing into two, one-half remaining with the antenna, the other half passing to roof of stomodæum. Note large mass of anterior endoderm.

Fig. 27.—Transverse section passing through labral lobes and roof of stomodæum. Stomatogastric ganglion shown in stomodæal roof.

Fig. 28.—Transverse section through stomodæal region. Tritocerebral ganglia are giving rise to subœsophageal commissure.

Fig. 29.—Transverse section, to show formation of supra-œsophageal commissure.

PLATE 5.

FIG. 30.—Transverse section through nerve cord after separation from hypodermis. Transverse commissure formed.

FIG. 31.—Longitudinal section, to show segmental arrangement of neuroblasts following segmentation of the ectoderm.

FIG. 32.—Transverse section (right half) through region of junction of stomodæum and mid-gut. Antennary mesoderm lies above the stomodæum and is enclosing the stomatogastric nerve. Visceral muscles are differentiating into circular and longitudinal fibres.

FIG. 33.—Longitudinal section through the anterior endoderm mass, showing inward migration of the endoderm strand.

FIG. 33, *a*.—Transverse section through the endoderm rudiment at the beginning of the formation of endoderm strands.

FIG. 34.—Longitudinal section through stomodæum. Note preoral mesoderm, anterior endoderm, subœsophageal body (premandibular mesoderm).

FIG. 35.—Section through yolk, showing loose cells from the anterior endoderm mass lying between yolk cells.

PLATE 6.

FIG. 36.—Longitudinal section through proctodæum, showing endoderm continuing to be proliferated from inner end of proctodæum.

FIG. 37.—Transverse section (dorsal half) through midgut region after dorsal closure of the body wall. Note heart rudiments and pericardial strands.

FIG. 38.—Longitudinal section through stomodæum after nerve cord differentiation. Note subœsophageal body (single cell in this section) beneath inner end of stomodæum. Muscle layer on dorsal side of stomodæum.

FIG. 39.—Transverse section through prothoracic region just before final closure. Main organs now formed but heart still in form of two separate rudiments. Beneath stomodæum lies the subœsophageal body.

FIG. 40.—Transverse section through proctodæum showing invaginations to form malpighian tubules.

FIG. 41.—Longitudinal section through proctodæum, in same stage as shown in fig. 40.

PLATE 7.

FIG. 42.—Transverse section through stomodæum, to show junction of two heart rudiments in form of inverted gutter, before ventral closure to form a tube.

FIG. 43.—Transverse section through stomodæum. The antennary mesoderm is enveloping the frontal stomatogastric ganglion.

FIG. 44.—Transverse section through stomodæum. The cavity of the aorta round the frontal ganglion is forming between the conjoined masses of antennary mesoderm.

FIG. 45.—Transverse section through stomodæum showing completed cephalic aorta.

FIG. 46.—Transverse section through stomodæum, showing relation of stomodæum to aorta, mandibular gland and corpus allatum in complete embryo.

FIG. 47.—Transverse section through stomodæum, showing the aorta open ventrally to the hæmocœl.

FIG. 48.—Transverse section through developing œnocytes in post-spiracular body wall of the abdomen.

FIG. 49.—Transverse section through similar position to latter, in a later stage showing œnocytes lying within the hypodermis.

PLATE 8.

FIG. 50.—Transverse section through mandibular, maxillary and labial segments.

FIG. 51.—Transverse section through labral lobes and protocerebrum.

FIG. 52.—Transverse section through epipharynx, just before its fusion with the labrum.

FIG. 53.—Transverse section through the epipharynx in its primary bifid condition

FIG. 54.—Transverse section through the epipharynx, after fusion of its original two lobes and showing the portion of preoral mesoderm in association with it.

FIG. 55.—Longitudinal section through the stomodæum, showing secondary separation of preoral mesoderm into labral and epipharyngeal portions.

PLATE 9.

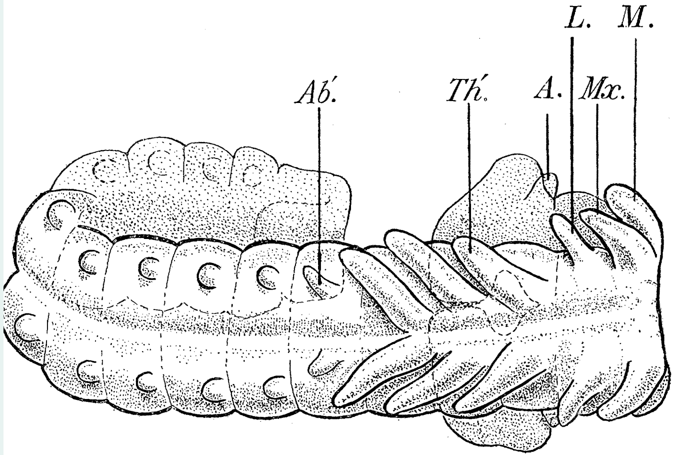
FIG. 56.—Drawing of three superimposed transverse sections through the head segments (right half), to show the places of origin of tentorial and mandibular apodeme invaginations and of the glands associated with maxillary and labial segments.

FIG. 57.—Transverse section (right half) through the head of the complete embryo, showing the large mandibular apodeme and the posterior tentorial invagination (at this stage posterior to the labium).

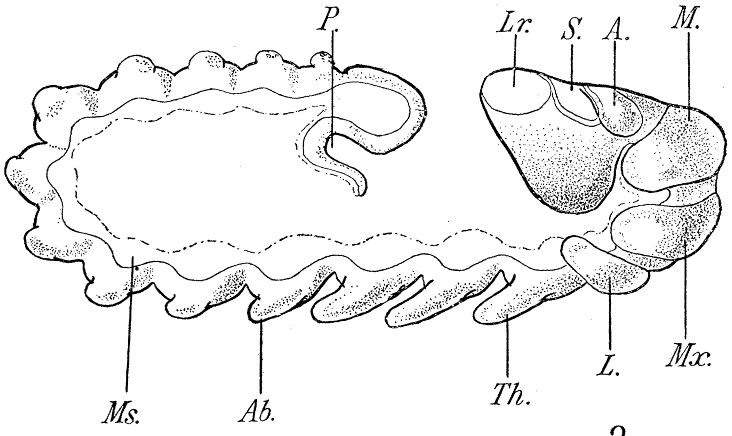
FIG. 58.—Transverse section (left half) through the head of the complete embryo, taken about 50 μ anterior to that of fig. 57, showing anterior tentorial arm, premandibular invagination and mandibular apodeme.

FIG. 59.—Transverse section through maxillary and labial region of embryo, showing the glands associated with the mandibular, maxillary and labial segments.

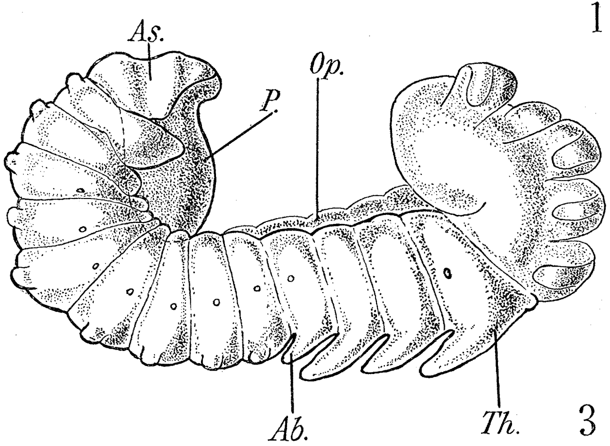
FIG. 60.—Transverse section through the head of the complete embryo (right half), in the maxillo-labial region, showing the external opening of the mandibular apodeme and attachments of the elevator and extensor muscles to the base of the mandible.


Ab'.
Th'.
A.
L.
M.
Mx.

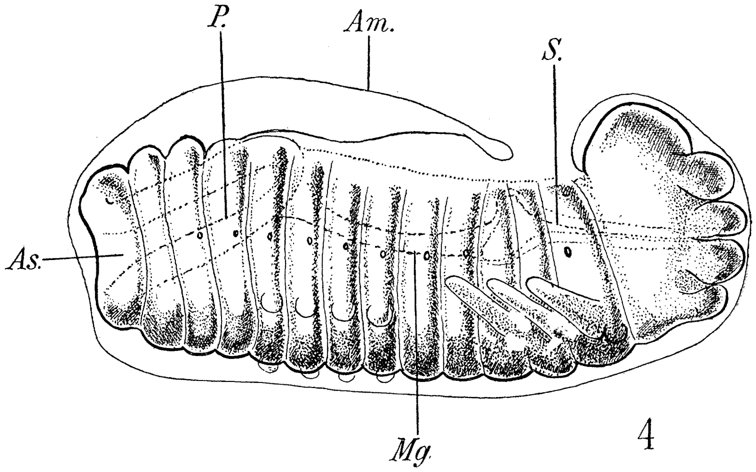
1


P.
Lr.
S.
A.
M.
Mx.
L.
Th.
Ab.
Ms.

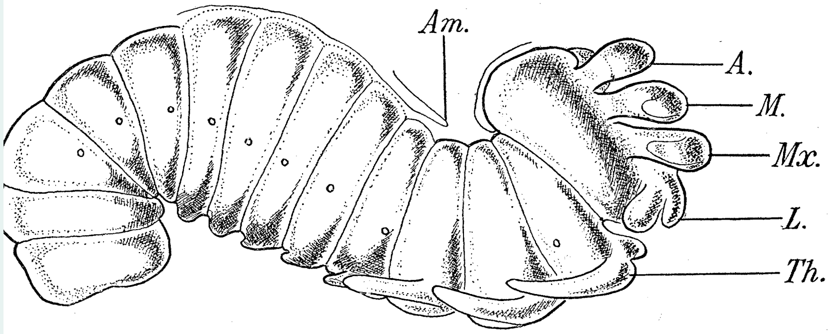
2


As.
P.
Op.
Ab.
Th.

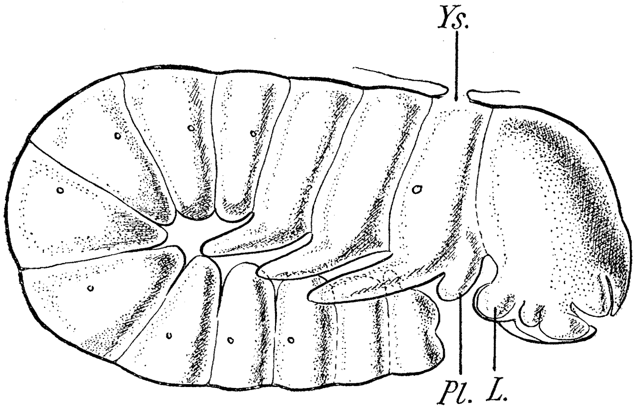
3


P.
Am.
S.
As.
Mg.

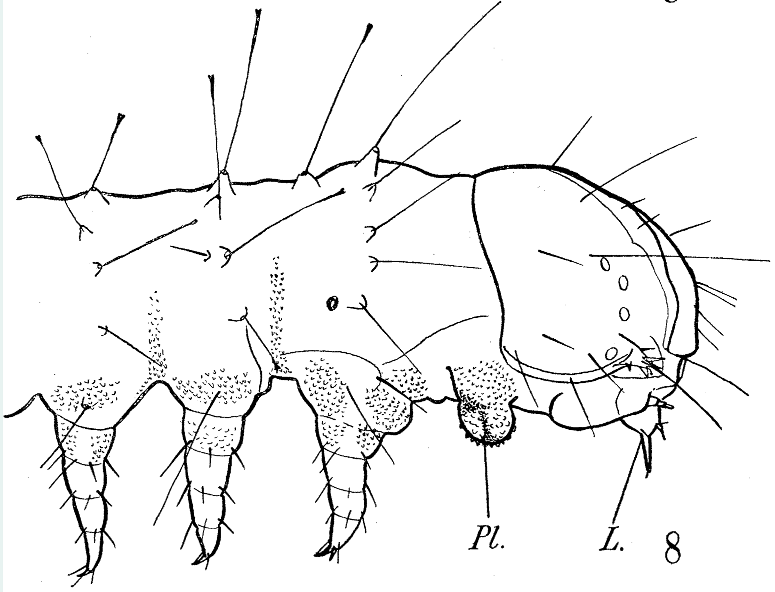
4


Am.
A.
M.
Mx.
L.
Th.

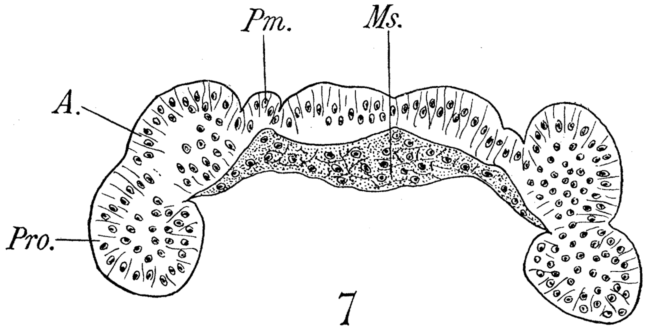
5


Ys.
Pl.
L.

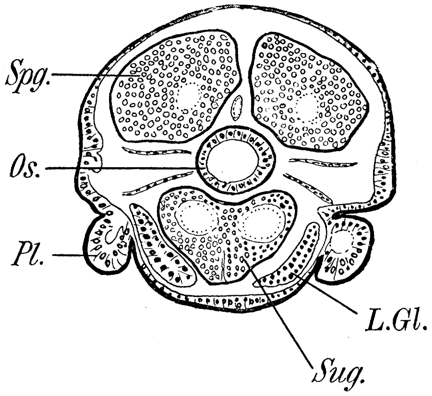
6


Pl.
L.

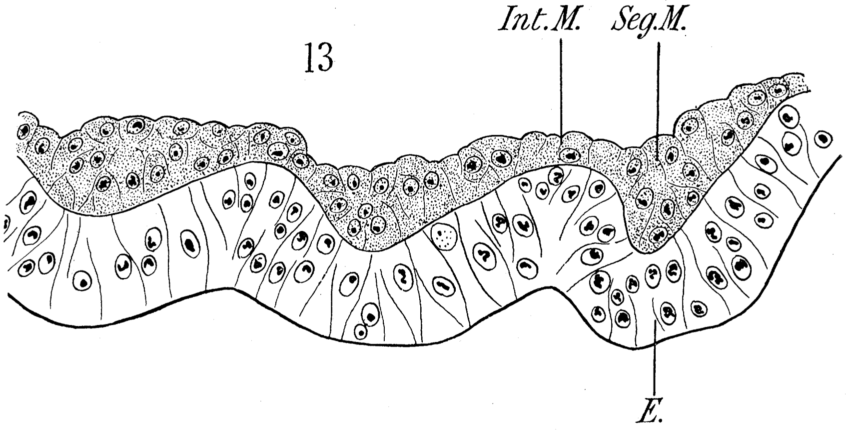
8


Pm.
Ms.
A.
Pro.

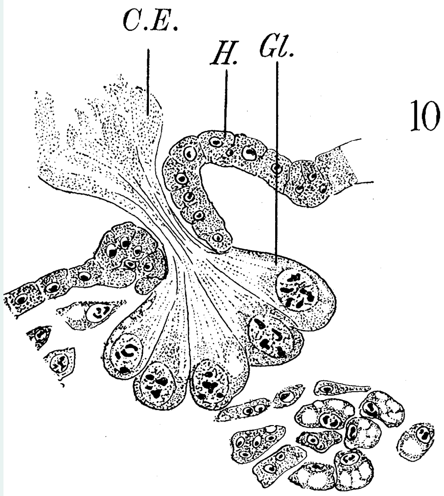
7



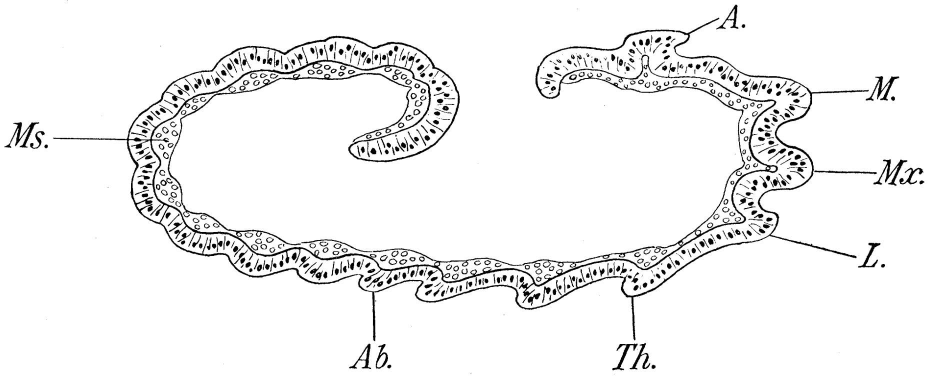
9



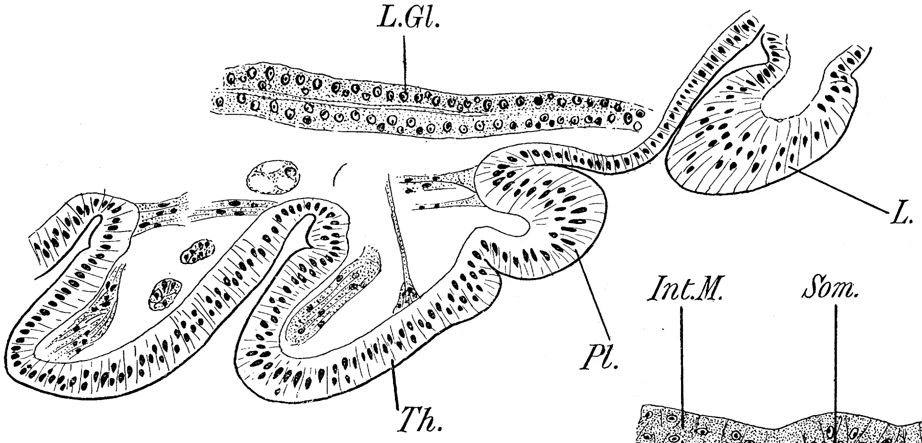
13



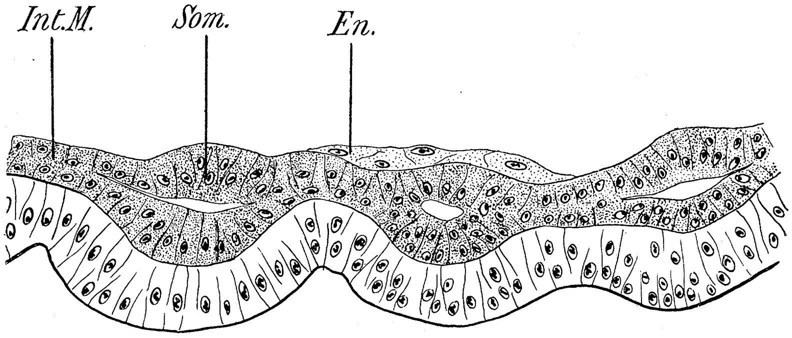
10



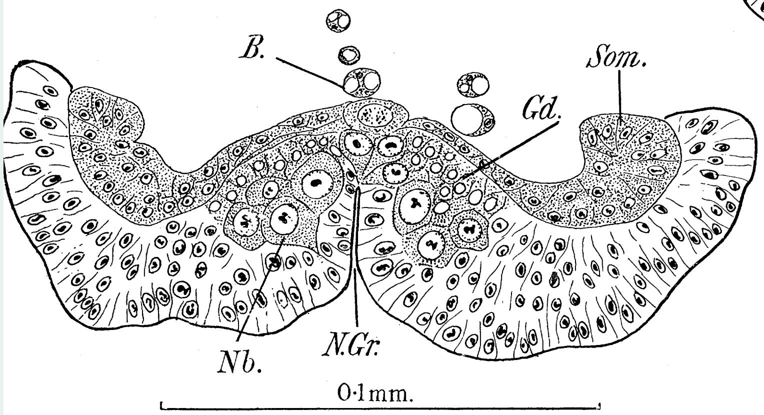
14



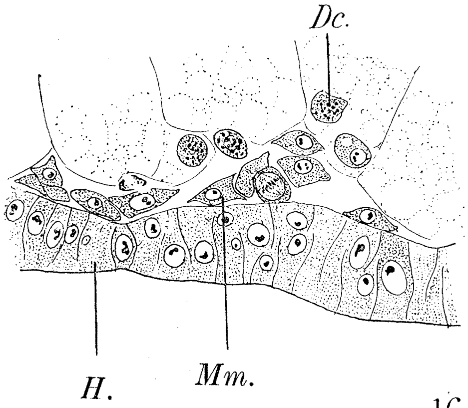
11



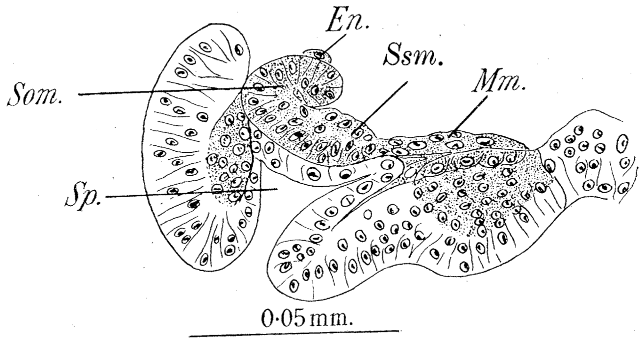
15



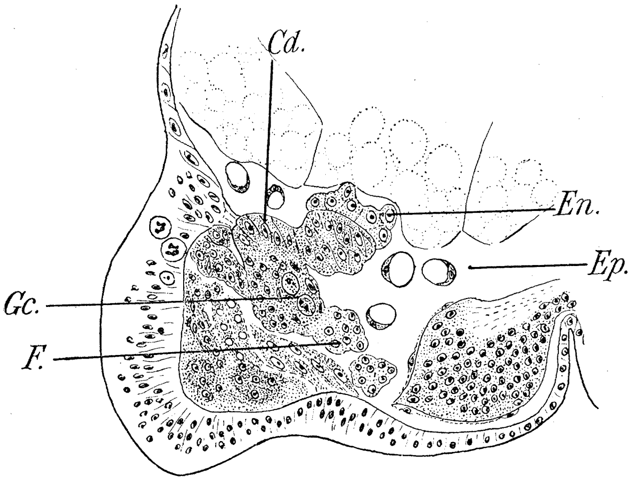
12



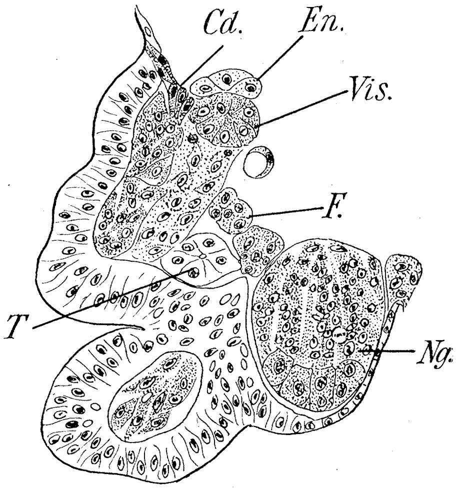
16



20

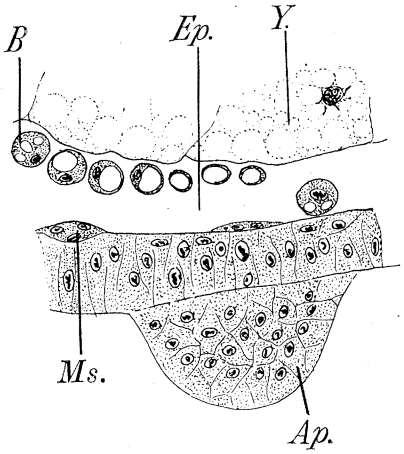


17

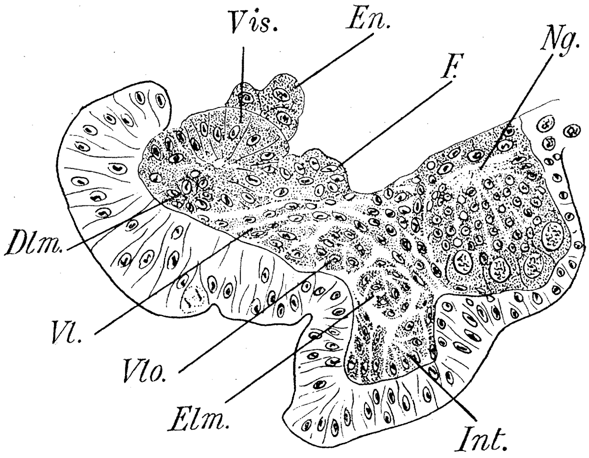


21

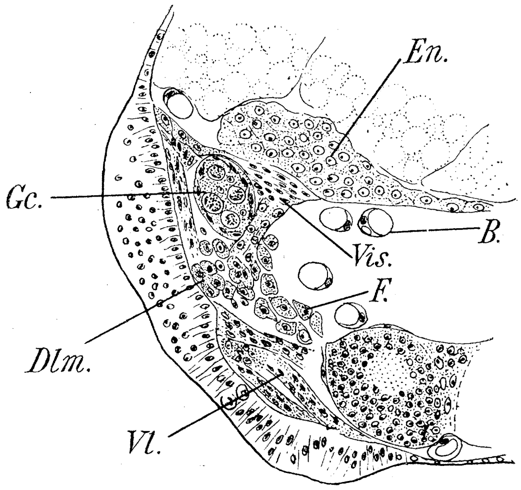
18

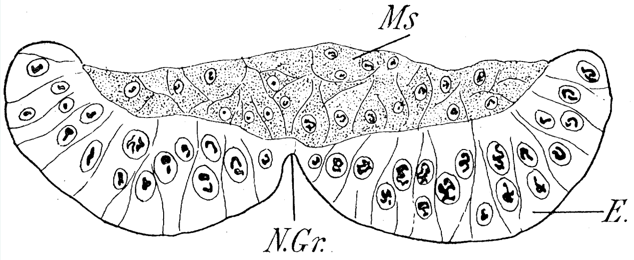


19

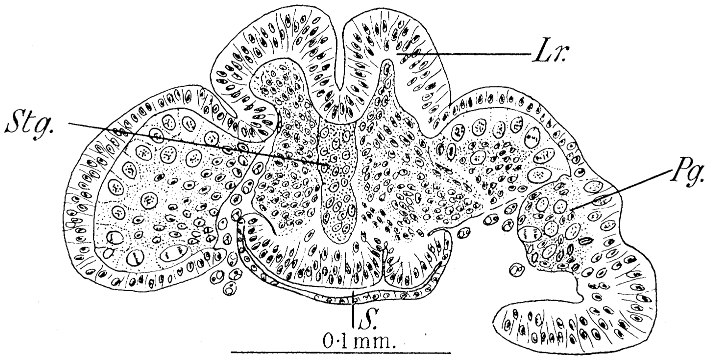


22

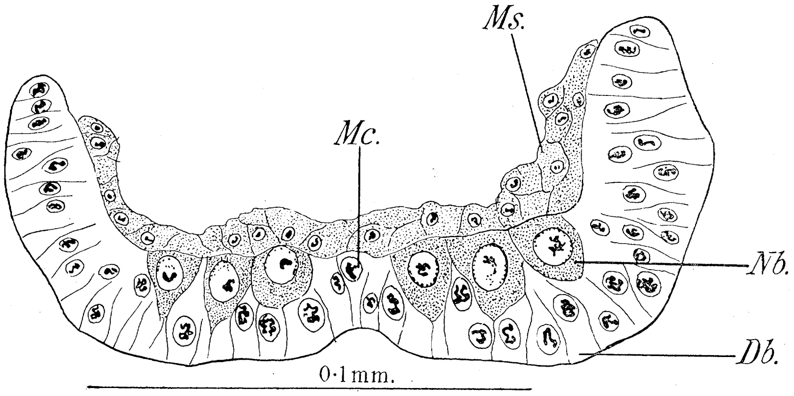




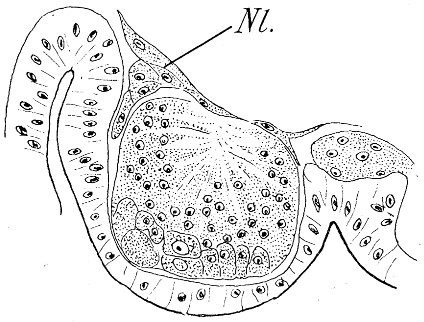
23



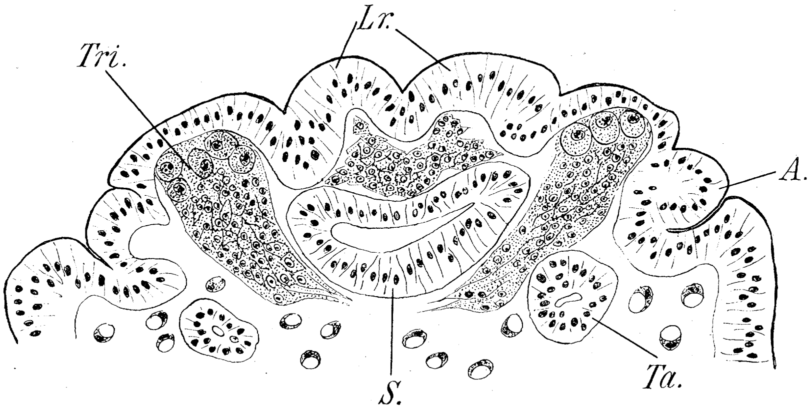
27



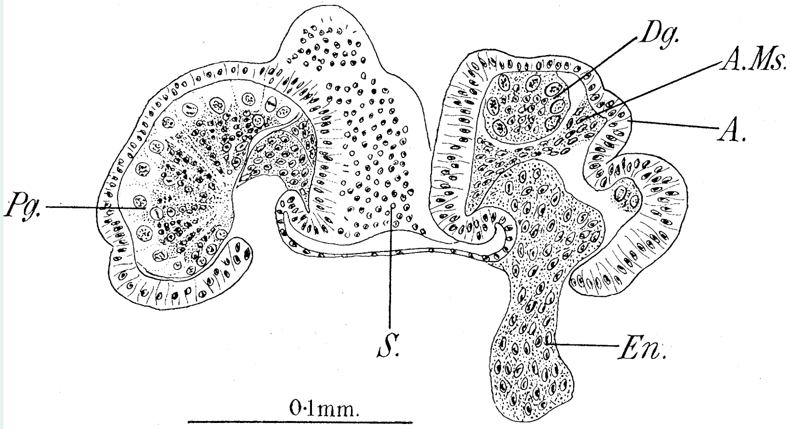
24



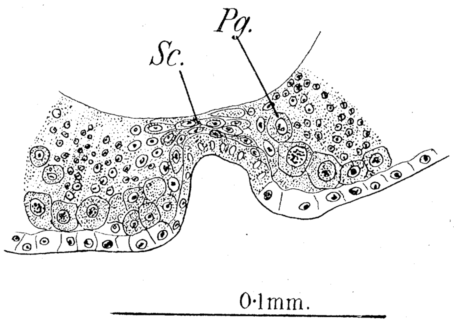
25



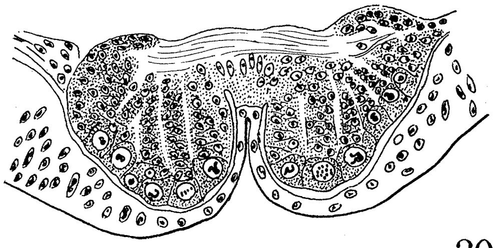
28



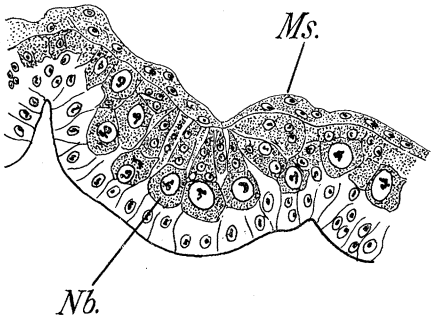
26



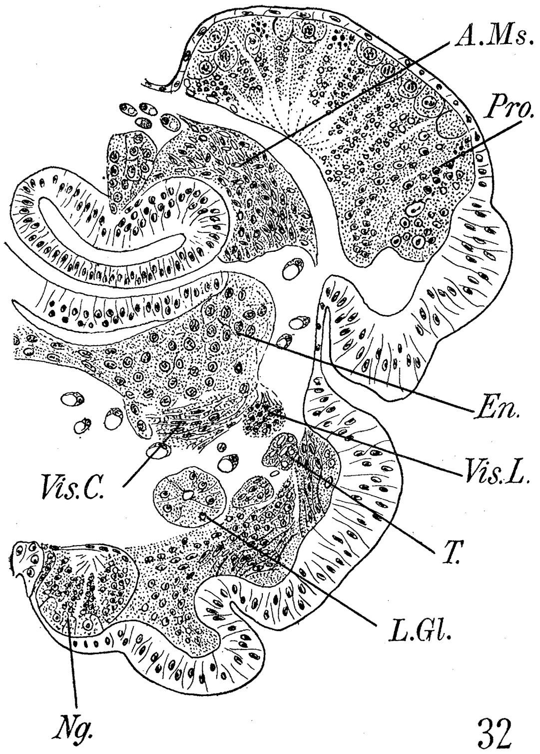
29



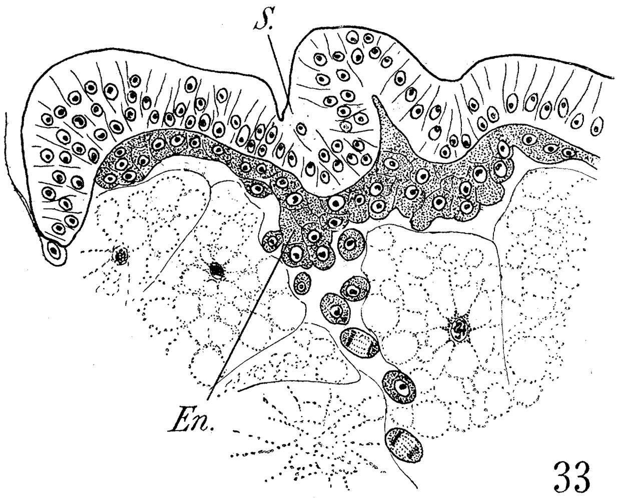
30



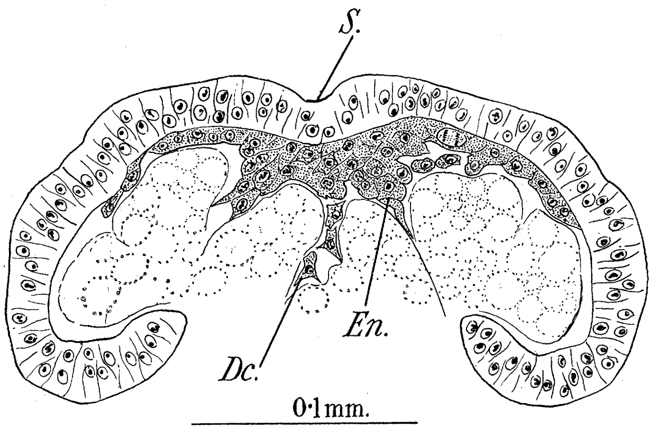
31



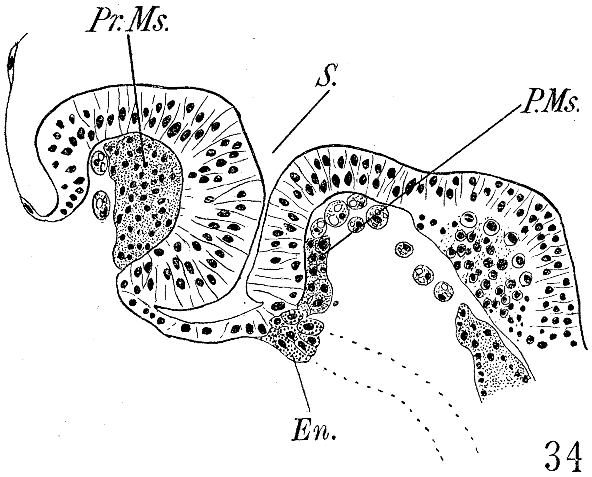
32



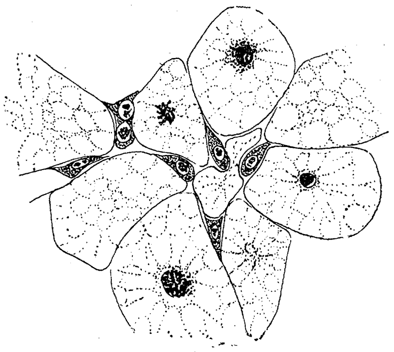
33



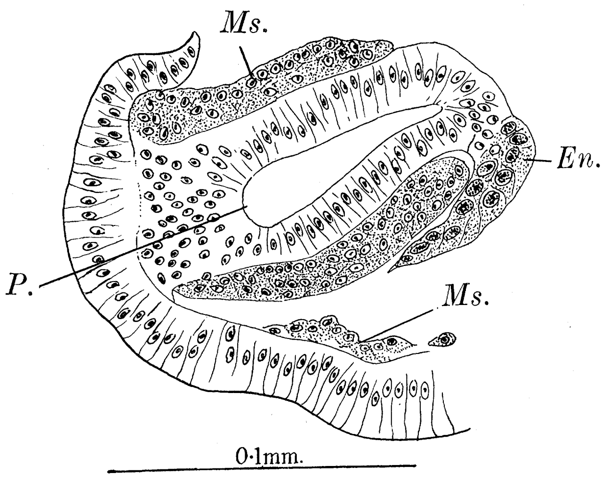
33*a*



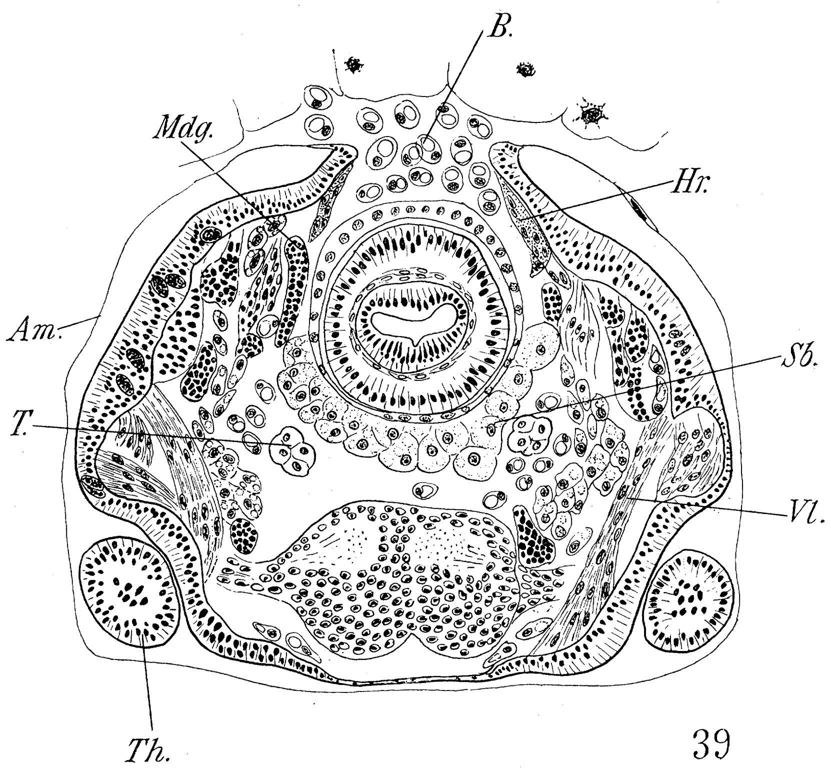
34



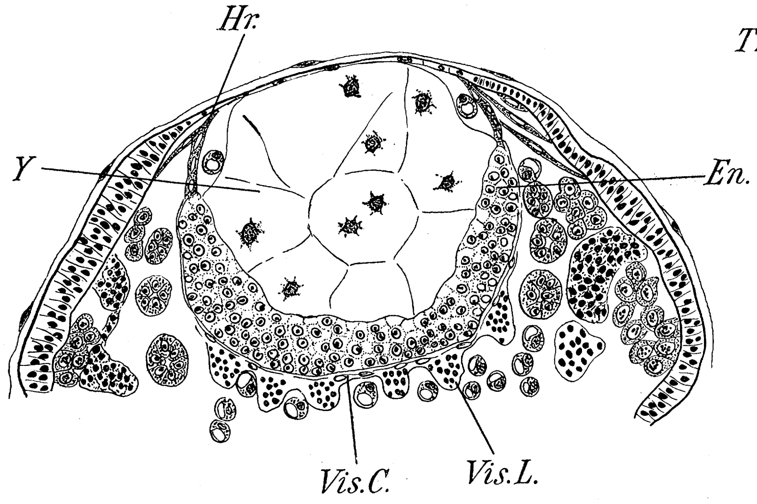
35



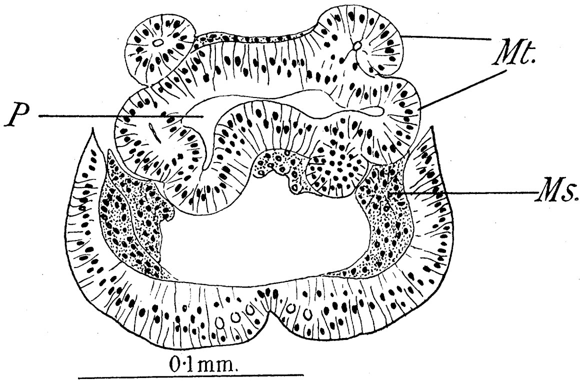
36



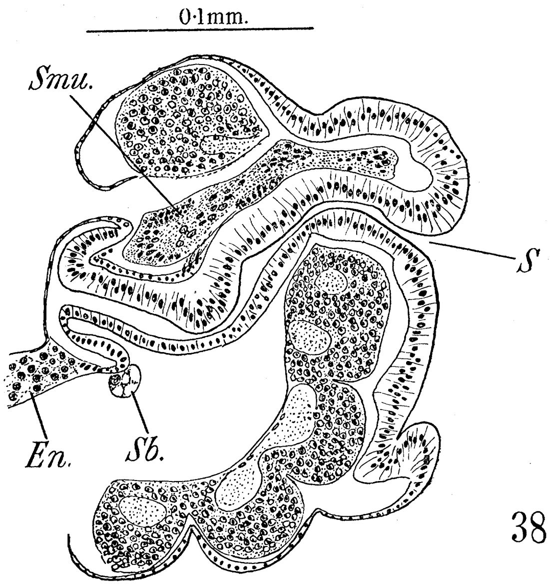
39



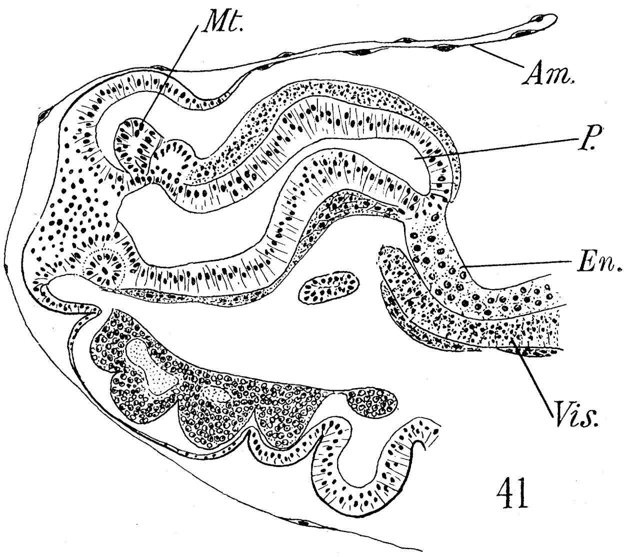
37



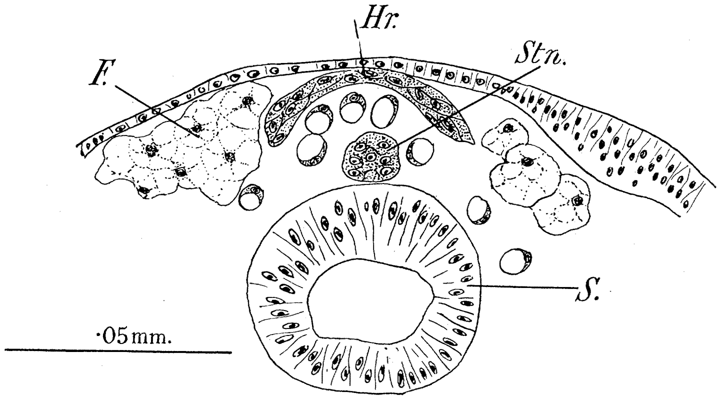
40



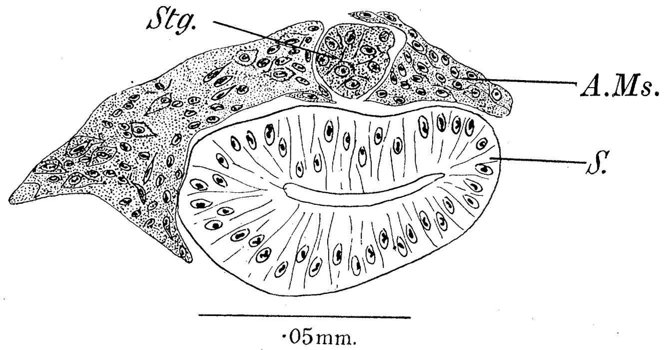
38



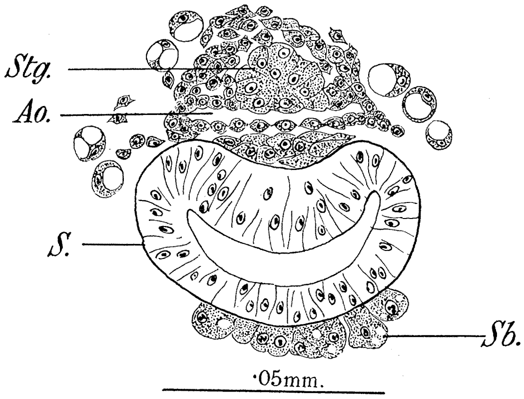
41



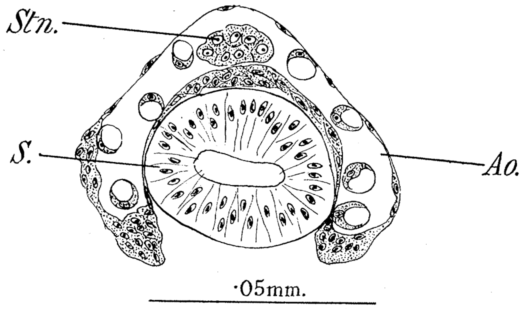
42



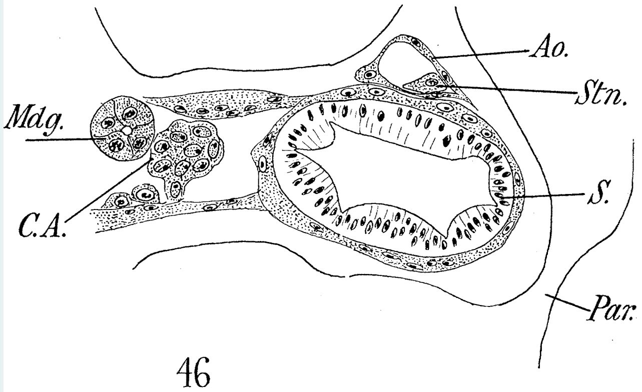
43



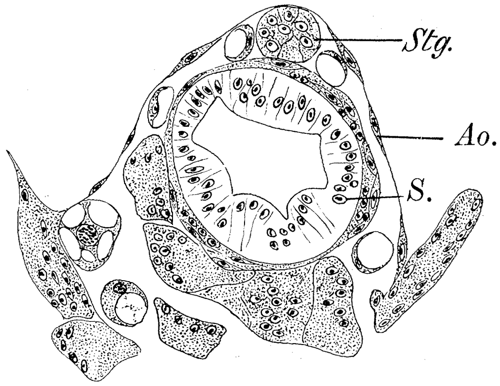
44



45

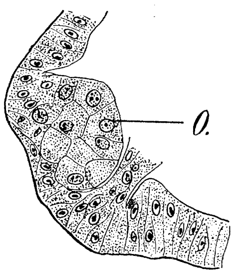


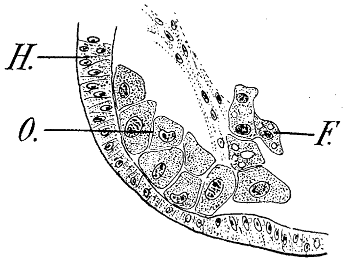
46



47

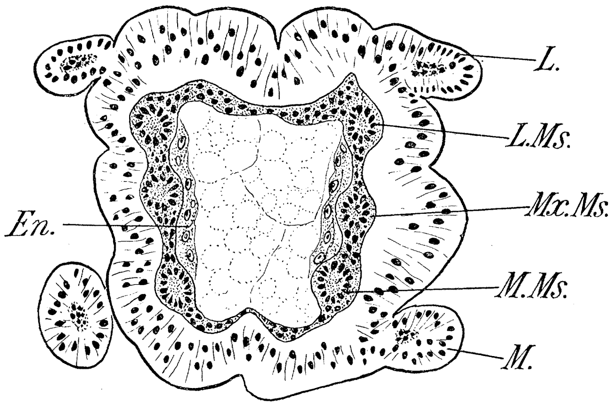
48



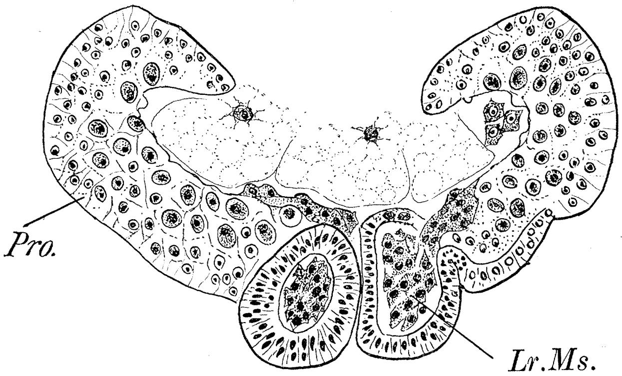


49

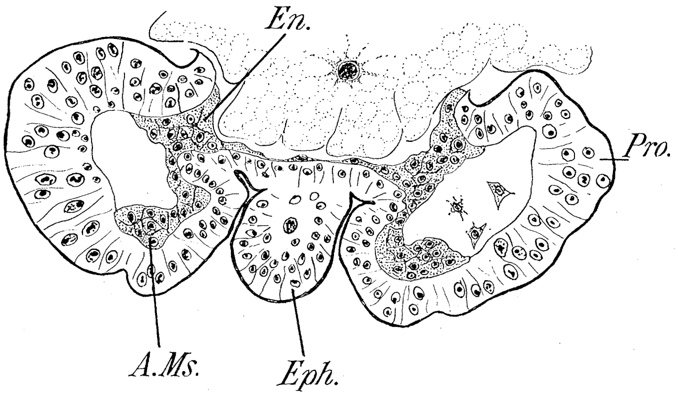
·05 mm.



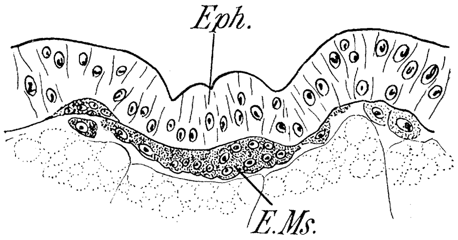
50



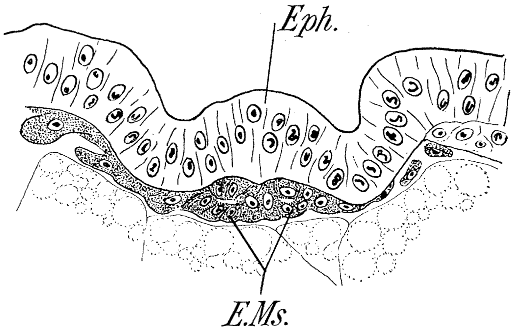
51



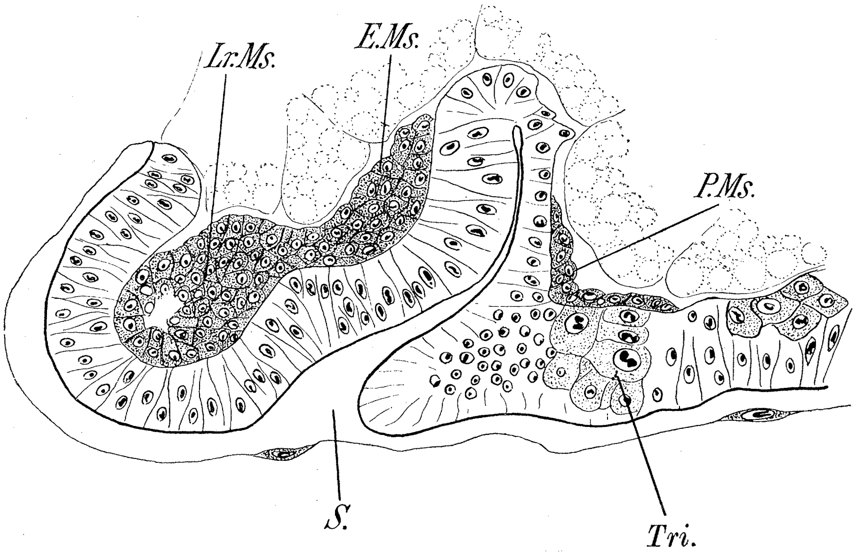
52



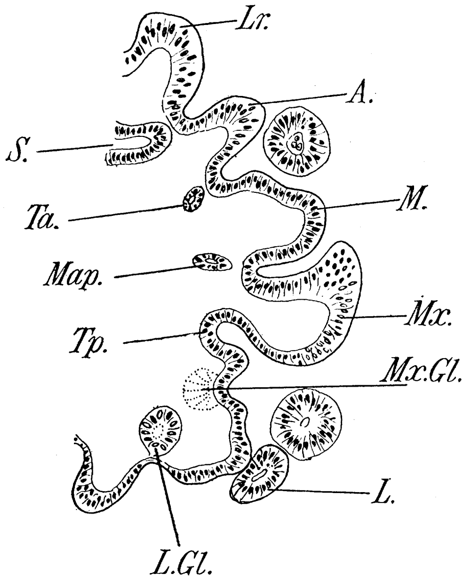
53



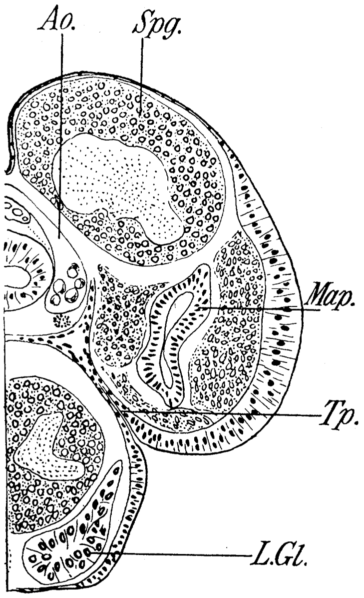
54



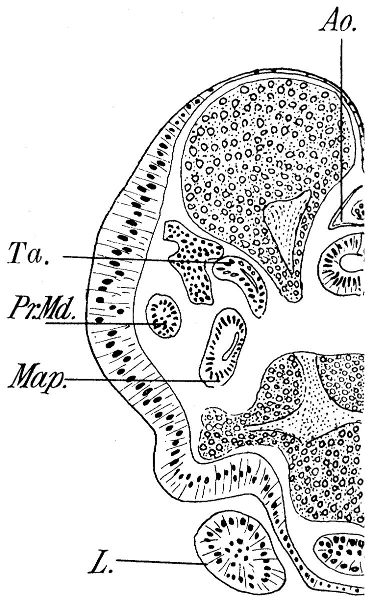
55



56



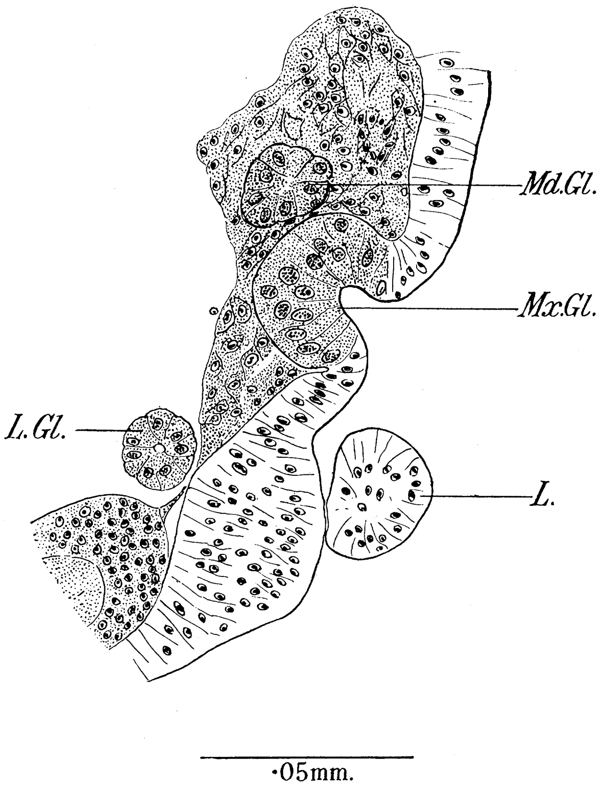
57

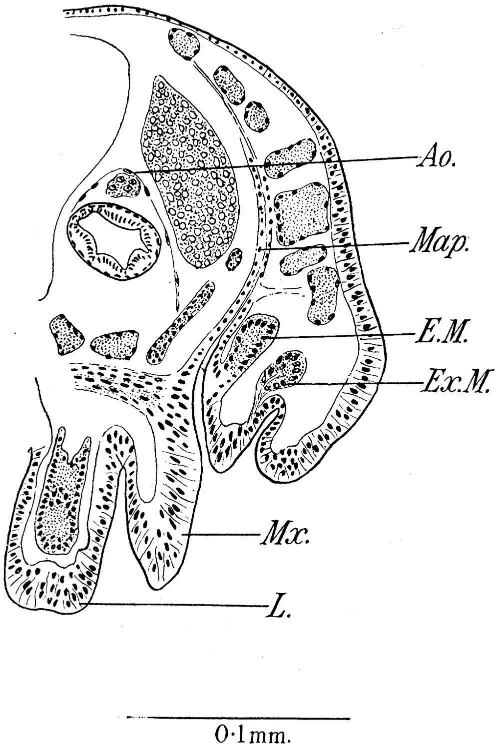


0·1mm.

58

59





60