with The author's Compliments

A CONTRIBUTION

TO

INSECT EMBRYOLOGY.

AN INAUGURAL DISSERTATION

FOR THE

DEGREE OF DOCTOR OF PHILOSOPHY,

PRESENTED TO THE

FACULTY OF CLARK UNIVERSITY.

May 10, 1892.

BY

WILLIAM MORTON WHEELER.

Reprinted from JOURNAL OF MORPHOLOGY, Vol. VIII., No. 1.

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The very primitive and synthetic character of the Orthoptera has long been recognized by systematists and comparative anatomists, but the full importance of the group from an embryological standpoint has been but little appreciated, owing to the meagre and fragmentary nature of the observations hitherto published. For this reason I have made the Orthoptera the starting point of my studies, with a view to determining their relations, on the one hand to the Apterygota and on the other to the higher Pterygote orders. Only a portion of the evidence bearing on these relationships is presented in the following paper ; a number of observations on the Malpighian vessels, corpus adiposum, œnocyte-clusters and abdominal appendages will be published as separate papers.

I have devoted more attention to *Xiphidium* than to other Orthoptera, partly because the Locustidæ occupy a somewhat central position in the order, and partly because this curious form exhibits in its embryogeny better than any other insect hitherto studied, the co-existence of certain very ancient with very modern characters.

My German co-workers in the field of insect development will probably regard my treatment of the literature as rather perfunctory ; but Prof. Graber, Dr. Heider and others have given from time to time such complete résumés of past and current literature that I feel justified in departing from the general custom. If I have failed to give credit where it is due,

I beg that this may be regarded as a fault of omission and not as a fault of commission.

I would express my sincere gratitude to Prof. C. O. Whitman for his kindly guidance and friendly counsel throughout the progress of my work in his laboratory at Clark University during the autumn and winter, and at the Marine Biological Laboratory during the summer months, of 1891 and 1892. I am also indebted to Mr. S. H. Scudder for the identification of several Orthoptera.

I. The Embryonic Development of the Locustidæ.

1. *The Oviposition of Xiphidium ensiferum*, Scud.

Xiphidium ensiferum, Scudder, a very common Locustid in Wisconsin and the neighboring states, deposits its eggs in the silvery napiform galls produced by *Cecidomyia gnaphaloides* (and perhaps allied species) on the low willows that abound in the marshy lands and along small water courses. I have found the insect ovipositing from the middle of August to the middle of September. It thrusts its ensate ovipositor in between the imbricated scales of the gall and places its eggs singly or in a more or less even row with their long axes directed like the long axis of the gall. The eggs are completely concealed by the scales, the overlapping edges of which spring back to their original positions as soon as the ovipositor is withdrawn. The number of eggs deposited in a gall varies greatly: sometimes but two or three will be found; more frequently from fifty to one hundred; in one small gall I counted 170 and I have opened a few which contained more. Sometimes as many as ten eggs will be found under a single scale; when this is the case, the eggs adhere to one another and are more or less irregularly arranged, as if two or three insects had in succession oviposited in the same place.

The *Cecidomyia* galls vary considerably in shape: some are long and more or less fusiform, others are spheroidal. In the former variety the scales are pointed and flat, while in the latter they are rounded and have their median concave portions less closely applied to the convex surfaces of the scales

which they overlap. These differences materially affect the eggs, for many of those thrust in between the closely appressed scales of the spindle-shaped galls are so much flattened as to be incapable of developing; on the other hand the eggs deposited in the more spacious interstices of the globular galls are usually in no wise injured. The two forms of gall do not always occur in the same locality and may be the productions of two distinct species of *Cecidomyia* or of one species on different willows. The Locustids, however, seem to show no preference for the globular galls.

The galls of *Cecidomyia*, being essentially stem-galls, do not drop to the ground in the autumn like the various leaf-galls on the willows, but persist through several seasons. Although the insects are not averse to ovipositing in the fresh galls, they nevertheless seem to prefer these blackened and weather-beaten specimens, probably because their scales are more easily forced apart.

I have called attention to the fact ('90[b]) that *X. ensiferum* departs widely in its habits of oviposition from its congeners, several of which are known to lay their eggs in the pith of easily penetrated twigs, like the species of the allied genus *Orchelimum*. *X. ensiferum* has evidently found it of great advantage to make use of the galls so abundant in its native haunts. So recent may be the acquisition of this habit, that on further investigation some females may, perhaps, even now be found to have a tendency to oviposit, like *Conocephalus ensiger*, between the root-leaves and stems of plants, or even in the plant tissues. It still occasionally happens that the eggs are run through or into the tissues of the gall-scales, and not loosely deposited. The fact that the insects have not yet learned to distinguish the kind of gall best adapted to their purposes, lends some support to the view that it is not so very long since *X. ensiferum* agreed with its congeners in habits of oviposition.[1]

[1] In the vicinity of Worcester, Mass., I found galls very similar to those formed on the Wisconsin willows. They contained a few slender yellow eggs, smaller than those of *X. ensiferum*. As this species does not occur in New England I conclude that these eggs were probably deposited by the very common *X. fasciatum*, De Geer.

2. *The Formation of the Embryo and its Backward Passage Through the Yolk.*

a. SURFACE CHANGES.

The sub-opaque, cream-colored egg of *Xiphidium* is elongate oval, 3–5 mm. long and 1 mm. broad through its middle. One of its poles is distinctly more attenuate than the other, and there is a faint curvature in the polar axis which causes one side of the egg to be distinctly convex and the other distinctly concave. The broader pole is the posterior, and is the first to leave the vagina during oviposition; the attenuate pole is, therefore, the anterior. In the galls the eggs stand with their attenuate poles pointing upwards. The convex face of the egg is the ventral, the concave face the dorsal region. Inasmuch as the egg undergoes no change in shape during development, it is easy to orient the embryo in its different stages. This is of considerable importance, as will appear from the sequel.

The yolk is pale yellow and very similar in constitution to the yolk of other Orthopteran eggs. It is enclosed by a thin leathery chorion which suddenly becomes transparent on immersion in alcohol. When dry it is white, and the creamy color of the egg is due to the yellow yolk shining through. As in *Blatta*, the chorion is the only envelope of the freshly laid egg; what I described in a former paper ('90b) as the vitelline membrane is in reality comparable to a "Blastodermhaut" as I shall point out.

The chorion varies somewhat in thickness at different points in the egg, being 11μ towards the middle and 19μ at the poles. It is quite elastic and when cut curls in at the edges. Its inner surface is very smooth, while outwardly it is covered with round or oval projections which measure about 3.7μ in diameter. They are flattened at their summits and are placed so closely together that only narrow channels run between them and give the chorion the appearance of being covered with a fine net of nearly uniform meshes. On closer examination it is seen that the projections are arranged in hexagonal groups. These are very distinct at either pole but fade away

on the median portions of the egg till they become very difficult to resolve. They evidently coincide with the areas covered by the polygonal cells of the follicular epithelium.

No traces of micropyles could be found. Their absence in *Xiphidium* is of interest, since Leuckart ('55) long since described and figured them in several European Locustidæ (*Meconema*, *Decticus*, *Locusta*, *Ephippigera*). In these genera they consist of funnel-like perforations on the ventral surface of the chorion either near the anterior pole or nearer the middle of the egg.

The preblastodermic stages were not studied. They probably resemble the corresponding stages of *Blatta*, of which I have given a detailed account in a former paper ('89).

When fully formed the *Xiphidium* blastoderm, like that of *Blatta*, consists of a thin sheet of cells, that have in part reached the surface from the interior of the egg, and are in part derived from these centrifugal cells by tangential division after their arrival at the surface. Numerous cells — the future vitellophags — are to be found at different points in the yolk. Whether they are derived from the incompleted blastoderm by centripetal division, or are inhibited before reaching the surface, my limited observations will not permit me to decide.

The cells forming the blastoderm are polygonal, much flattened and of uniform size and distribution. Those on the center of the convex, or ventral face of the egg soon begin to change their dimensions; from being broad and flat, they become more nearly cubical, their lenticular nuclei again assuming the spherical or oval shape which they had in preblastodermic stages. These changes take place over a limited and somewhat oval area and result in the formation of the ventral plate. The few eggs that I have been able to find in the very first stages after the completion of the blastoderm leave me in some doubt as to the exact process whereby the embryo is established. I am satisfied, however, that the thickening and narrowing of the individual blastodermic cells does not take place simultaneously over the whole ventral plate area, but that there appear, as in the crustacean egg (*e.g.* *Astacus*, *Homarus*), several discrete centres about which the

cells are at first more closely aggregated. The spaces between these centres are subsequently filled in by tangential cell-divisions. Of such centres I can distinguish four: two of them, the precursors of the procephalic lobes, are paired, while the other two form respectively the growing caudal end of the ventral plate and what I shall call the indusium.[1] The indusial centre, which does not make its appearance till a short time after the other centres are formed, does not join the body of the embryo till after the spaces between the procephalic and caudal centres are filled in. This is distinctly seen in Fig. 1 (Stage A) where the somewhat T-shaped embryo is already established and distinctly marked off, at least posteriorly, from the undifferentiated blastoderm. The nuclei of the blastoderm are as yet no larger than the nuclei of the ventral plate. Numerous caryokinetic figures in all parts of the embryo bear witness to active cell proliferation. No such figures were to be seen in the extra-embryonal blastoderm during and after this stage. The ventral plate including the indusium is scarcely a fifth as long as the egg, being much smaller in proportion to the size of the yolk than in some other Orthoptera (*Blatta*, *Gryllotalpa*).

The blastopore is seen in the stage figured as a very narrow but distinct groove extending from the oral region to the caudal end of the embryo, where it bifurcates before its termination. The infolded cells give rise to the mesoderm and also, I believe, to the entoderm.

In *Xiphidium* the three folds that form the amnion and serosa arise like their homologues in *Blatta*. The first appears as a crescentic duplication surrounding the caudal end; thence it grows forward and after enveloping the whole postoral portion of the embryo coalesces with the two head-folds, each of which arises from the edge of a procephalic lobe. The progress of the anal fold is shown in Fig. 2 (Stage B) Pl. I. Although agreeing in its main features with what has been described for most insect embryos, the process of envelope-

[1] In a preliminary note ('90c) this structure was called the præoral plate (Präoralplatte). Many reasons have led me to abandon this term together with others referring to the parts of the organ in its subsequent development.

formation in *Xiphidium*, is, nevertheless, peculiar in two respects: first, the envelopes are so closely applied to the germ-band that in surface view their advancing edges can be detected only with difficulty, though they may be distinctly seen in sections; second, the point of closure of the envelopes is situated further forward on the head than in *Blatta*, *Hydrophilus*, *Doryphora*, etc. This I infer from an embryo, which I figure (Fig. 15. Pl. II.) Here the cells and nuclei of the amnion and serosa have become much larger than the cells and nuclei of the embryo. The edges of the folds are unusually distinct and enclose a circular space through which the oral and præoral regions are clearly visible. On the median anterior edge of the head the amnion and serosa are completely interrupted. In no other insects have I found the envelopes lacking on the anterior edge of the head in so late a stage. This fact is probably significant when taken in connection with changes about to occur in front of the head.

The wide procephalic lobes are succeeded by the strap-shaped body In this a number of segments have made their appearance. These are in order from before backwards: the mandibular (*md. s*), the first maxillary (*mx. s*^{1}), the second maxillary, (*mx. s*^{2}) the three thoracic (*p. s*^{1}-*p. s*3), and the first abdominal (*a. s*^{1}). Further back lies a small segment which is incompletely constricted off from the first abdominal and which I take to be the proliferating terminal segment, or telson. The seven segments depicted in the figure are undoubtedly definitive segments. The manner of their appearance will be clear from a glance at Fig. I. In A the ligulate part of the germ-band is seen to be faintly constricted at its base into two segments with indications of a third. In B, a slightly later stage, four definitive postoral segments are present, but a portion of the germ-band still remains unsegmented. This is, however, soon broken up into segments and we reach the stage in Fig. 15, Pl. II. It will be observed that the embryos in Fig. I are in many respects older than that in Fig. 15, Pl. II. The antennæ have made their appearance and the amnioserosal fold has closed completely. These embryos prove several points:—first, that the wave of metameric segmen-

tation passes from before backwards dividing the germ-band into 7 or 8 segments; second, that these segments are the definitive segments and not macrosomites, or complexes of definitive segments; and third, that there is considerable variation in the time when segmentation sets in. To these points I may add a fourth: segmentation appears first in the ectoderm and only somewhat later in the mesoderm.

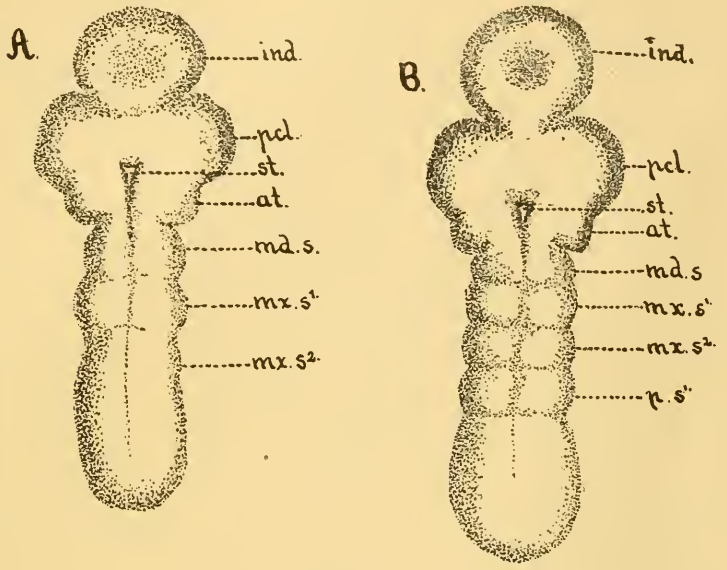

FIG. I.

A and B. Isolated embryos of *Xiphidium* in successive stages of metamerization. *ind.*, indusium; *pcl.*, procephalic lobe; *st.*, stomodæum; *at.*, antenna; *md. s.*, mandibular segment; *mx. s*¹, first; *mx. s*², second, maxillary segment; *p. s*¹, prothoracic segment.

The indusium (Fig. 15, *p. o.*) is still only a rounded thickening of the blastoderm. Its small deep cells are continuous through a zone of larger cells with the relatively very large and flat elements of the primitive cell-layer. Two broad and flat commissures appear to connect the organ with the procephalic lobes. Thus a small space containing a few larger cells is enclosed between the indusium and the head of the embryo. This space (*y*), seen as a clear spot in surface view, lies at the breach in the envelopes. In many embryos the indusium is

united with the head of the embryo (Fig. I, A and B) before the stage of Fig. 15 and soon after this stage is, I believe, normally united with it. This union is probably purely mechanical — the organ remaining at its place of origin on the surface of the yolk, while the embryo lengthens till its head unites with the posterior end of the organ. This union is of brief duration as is seen in Fig. 3 (Stage C).

During this stage the caudal tip of the embryo shows a tendency to bury itself in the yolk. The amnion and serosa, hitherto closely applied to each other, now separate at the caudal end, where, as I have said, they first arose as a crescentic fold. Soon the tendency to enter the yolk becomes more pronounced so that the tail curls back and leaves the ventral face of the egg. Meanwhile the remainder of the embryo moves down the ventral face a short distance, thus pushing its tail still further into the yolk and causing the separation of the envelopes to advance still further headwards. The indusium does not accompany the embryo in this movement, but remains nearly or quite stationary; consequently the head gradually separates from the organ till it is connected only by means of a slender band of cells in the median line. (Fig. 3 and Fig. 16.) This link soon ruptures and the indusium is set adrift from the embryo, or, more precisely, the embryo is set adrift from the indusium. (Fig. 4, Stage D.) In profile the embryo now resembles the small letter j, — the dot being supplied by the isolated indusium.

Important changes begin to affect the indusium during or more frequently just after its separation from the embryo. The closely packed cells at the periphery, as indicated by their nuclei, begin to arrange themselves radially (Fig. 16). Some of the large nuclei of the serosa may be seen encroaching on the edges of the disk from all sides, leaving only the median portion free. Sections show that the organ is now forming an amnion like that of an embryo. In the middle of the disk appear several shrunken but distinctly defined nuclei which are proved by focusing to be confined to the surface of the organ.[1]

[1] Only four of these peculiar bodies are represented in the figure (*nn*); there were several others in the same preparation, but for the sake of clearness I have omitted them in the drawing.

The serosal fold continues to advance from all sides till the organ is entirely covered. Viewed from its ventral surface the egg now has the appearance of Fig. 4 (Stage D). Here the indusium is cordate in outline and somewhat larger than usual. Of the abdomen only the two basal segments still remain on the ventral face of the egg ; the remaining segments curl back into the yolk.

During this and the two preceding stages the cephalic and thoracic appendages have become distinctly established as rounded lateral outgrowth of their respective segments. The antennæ (*at*) originate as lobular outgrowth from the posterior edges of the procephalic lobes. They are distinctly postoral in origin. The margins of the triangular oral orifice are somewhat swollen; the anterior edge, where the labrum is about to appear, is cleft in the median line (Fig. 16). The three thoracic segments are very slightly or no broader than the two maxillary segments. The appendages of these five segments are also alike in size, shape, and position. In very early stages of other insect embryos, even before the amnion and serosa are fully formed, the thoracic become broader than the maxillary segments, and the legs, as soon as they appear, may be readily distinguished from the two pairs of maxillæ by their greater size and prominence. The Locustid embryo, therefore, has even a stronger tendency to revert to annelid-like or myriopod-like ancestors than is apparent in any of the other insects whose ontogenies have been investigated.

The mandibular segment of *Xiphidium* like that of other insects, is somewhat retarded in its development. Between this and the antennary segment careful study of sections and surface preparations reveals the presence of another segment, shown very distinctly in outline in Fig. 16 (*tc. s.*). This is no other than what I have called the intercalary segment in *Doryphora*. It is the tritocerebrum of Viallanes ('90[a], '90[b]).

The embryo continues to move back into the yolk, following the curved path established by the inflexion of the posterior segments till its tail is finally arrested by striking the serosa on the dorsal surface. At this time the embryo has the form of an arc subtending the dorsoventral diameter of the egg.

Returning to consider the indusium, we find that it begins to increase in size before the embryo's head leaves the ventral face. The organ stains much less deeply, and even in surface view its expansion may be seen to be due to a flattening of its component cells. In Fig. 5 (Stage E) is represented an embryo merged in the yolk up to the first maxillary segment. The indusium extends around on either side nearly to the middle of the lateral face of the egg. Either the transition of the embryo takes place rapidly or the organ changes very gradually, for the latter is in about the same stage after the embryo has become established on the dorsal surface. The manner in which the expansion of the indusium is brought about will be apparent when I come to describe its structure in sections.

b. THE INDUSIUM IN SECTION.

As will be seen from the preceding account, the indusium is simply a circular thickening of the blastoderm, situated in the median line, between and a little in front of the procephalic lobes. It does not arise as a part of the ventral plate but as a separate centre which is at first merely a cluster of blastoderm cells that have changed from the pavement to the cubical or columnar type. This centre is further increased in breadth and thickness by caryokinesis. In the earliest stages examined, sections of the organ show the same cell-structure as sections of the procephalic lobes.

Median longitudinal sections of the embryo in Stage C are interesting as showing the relations of the indusium to the embryo and its envelopes. I reproduce such a section in Fig. 21, Pl. III. Here the organ (*p. o.*) appears as a large flattened cell-aggregate somewhat thinner in the centre than nearer its periphery. Owing to the shape of the mass, the median cells, as indicated by their nuclei, are arranged with their long axes perpendicular to the flat outer surface of the organ, while the cells of the thickened lateral portions become gradually more oblique till those on the extreme periphery assume the same position as the serosa cells (*s.*). The nuclei are most frequently

situated at the inner ends of the cells so that masses of enucleate protoplasm are left at the surface. Posteriorly the organ is linked to the embryo by means of a few flattened cells. In the section two of these cells are seen at *z* differing in no wise from the serosal elements (*s.*) in front and on either side of the organ ; the upper cell passes directly into the serosa covering the embryo, while the lower abuts on the cells that form the transition from the ectoderm to the amnion. The ectodermal layer of the embryo (*ec.*) is nearly as thick as the indusium and of similar cytological structure. The beginning of the stomodæal invagination is shown at *o.*

The next section figured (Fig. 17 Pl. II) is from an indusium in a somewhat younger stage than that represented in surface view in Fig. 2. Being transverse the section shows an evenly convex outer surface, continuous with the surface of the serosa (*s.*) enveloping the yolk. The cell-contours are still visible and show that the cells constituting the median portion of the organ are polygonal. The nuclei of these elements are spherical or oval and contain one, or more rarely, two nucleoli besides the usual chromosomes. In the peripheral ring-shaped thickening the cells (*d.*) are larger and pyramidal or fusiform in outline, while their nuclei differ in no wise from the nuclei of the median cells. The serosal cells stain more deeply than the cells of the organ, as may be seen at *s* where a single cell overlaps the edge of the disk. This depth of color is apparently purely optical, being due to the greater size and flatness of the serosal nuclei. The walls of both the small polygonal and larger pyramidal elements fade away towards the surface, where the bodies of the different cells become confluent to form a homogeneous mass.

In this surface-mass of protoplasm which takes the normal pink stain in borax carmine, are to be found several of the peculiar nuclei, mentioned above as distinctly discernible from the surface (Fig. 16). They differ markedly in structure and appearance from the normal nuclei in the inner portions of the indusium as will be seen by comparing the cells of Fig. 24 with those in Fig. 23, both of which figures were drawn with a high power. The normal cells (Fig. 23) have spherical or

oval, evenly rounded nuclei with one or two nucleoli and their chromatin is distributed in what I take to be the typical resting reticulum. The caryolymph, or Kernsaft, is faintly stainable. On the other hand, in the nuclei of Fig. 24 the nuclear wall is very irregular, the caryolymph much more limpid and refractive and the chromatic reticulum has coarser meshes. The chromatic nodes of the reticulum are larger than in Fig. 23 and seem to be applied to the indentations of the nuclear wall. Nucleoli appear to be absent. These specialized nuclei also vary greatly in size. In a series of sections it is easy to find nuclei intermediate between the two extremes here described, being evenly rounded but with colorless caryolymph and coarse chromatic reticulum. A cluster of four such nuclei is shown at nn^2 Fig. 17. These intermediate forms, occurring as they usually do, between the normal and the modified nuclei may be taken to indicate that the nuclei of the extreme types are genetically connected. Some of the normal nuclei probably leave their respective cells in the median portions of the organ and move up into the syncytial protoplasmic layer, undergoing the modification in structure during their emigration. When they have reached their destination they are perhaps broken down and converted into protoplasm. Certain it is that later no traces of them are to be found in the indusium. I do not believe that I am here considering collapsed and distorted caryokinetic figures, as these delicate structures are quite faithfully preserved in eggs killed by means of heat. The distorted nuclei are not confined to the indusium but occur also in the ectoderm of the embryo itself.

When the organ has reached the state just described it usually separates from the head of the embryo; it may, however, remain attached for some time longer. Like the embryo it is now an isolated body lying on the yolk; but unlike the embryo it is still only a part of the serosal envelope (which is itself only the extra-embryonal portion of the blastoderm). The serosa is a closed sack enveloping the whole yolk and the indusium is simply a swelling at one point on its inner face. (Fig. II, A.) The process of envelope formation which now begins in the indusium is much less clear than the cor-

responding process already completed in the embryo. From among the numerous preparations which I have made I select for illustration one (Fig. 18) which seems to show the process clearly. In surface view the organ would appear as in Fig. 3. The spreading of the serosal cells over the edges of the disk from all sides is now seen to be due to a process of induplication, or folding. The circular fold is, of course, cut in two places in the median transverse section figured. It advances in such a manner as to leave the outer face of the indusium evenly rounded and undisturbed, the upper surface of the fold usually forming a continuous line both with the outer surface of the serosa and with the median still uncovered portion of the organ. The fold continues to advance from all sides till the layers of which it consists meet and become confluent in essentially the same manner as the folds that form the amniotic and serosal layers over the embryo proper. We now have three layers of cells. (Fig. 19.) The outermost layer, *s*, is the serosa which has everywhere the same structure and evenly envelops the whole egg, having been separated first from the embryo and now by a similar process also from the indusium (Fig. II, B). The innermost layer consists of the unchanged greater portion of the organ. The median layer, to judge from its component cells, seems to be derived exclusively from cells of the original body of the organ and not from the serosa. This layer is, therefore, like the amnion of the embryo proper, structurally more closely related to the body it envelops than to the serosa. Fig. 18 favors this conclusion, which presupposes that only the outer half of the circular fold is derived from the serosa, for in this section the lower and thicker layer of the fold on either side certainly consists of cells derived from the body of the organ. Even before the layers are fully formed the edges of the two-layered organ are sharp and somewhat irregular (Fig. 18), not rounded like the edges of the embryo when its amnion is completed. The whole organ still has essentially the same form that it had in the stage represented in Fig. 17.

It will be convenient to name the different layers of cells, thus far distinguished. For the amnion of the embryo proper

I shall retain the old name; the corresponding envelope of the indusium and the body of the organ will be designated as the outer and inner indusium respectively.

In by far the greater number of cases the process of

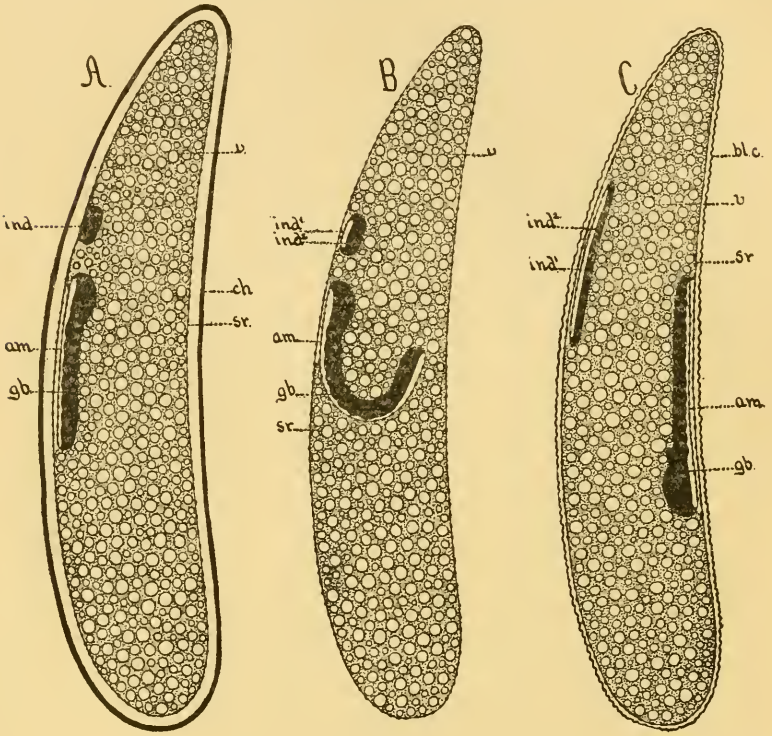

Fig. II.

Diagrams illustrating the movements and envelopes of the *Xiphidium* embryo. *A*, after the closure of the amnioserosal folds; *B*, during the embryo's passage to the dorsal surface; *C*, just after the straightening of the embryo on the dorsal surface. *ind.*, indusium — afterwards forming *ind*¹, the inner, and *ind*², the outer indusium; *ch.*, chorion; *sr.*, serosa; *am.*, amnion; *gb.*, germ-band; *v.*, yolk; *bl. c.*, Blastodermhaut.

envelope formation over the indusium is much obscured by rapid slurring. In fact the whole process has frequently the appearance of being due rather to a shifting and migration of cells than to the formation of true folds. The cells of the serosa seem to creep over the disk while the cells forming the

edge of the organ itself appear to creep along under and a little in the rear of the advancing serosal elements. I cannot here go into greater detail without unduly increasing the number of my figures. Nor is it necessary, since it will, I believe, be

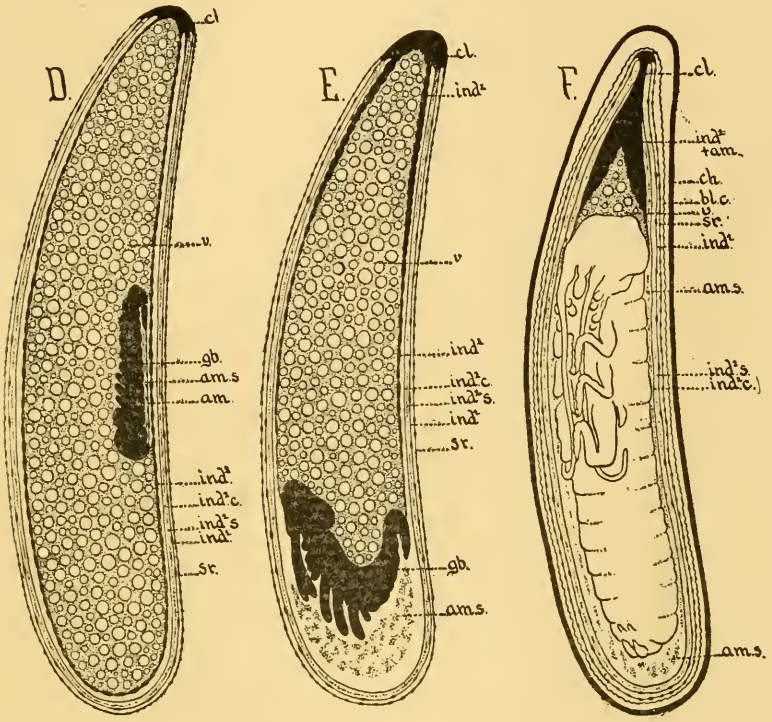

FIG. II.

Diagrams illustratlng the movements and envelopes of the *Xiphidium* embryo. *D*, the stage of the shortened embryo on the dorsal yolk ; *E*, embryo returning to the ventral surface ; *F*, embryo nearly ready to hatch. *ch.*, chorion ; *bl. c.*, Blastodermhaut ; *sr.*, serosa ; *ind*¹, outer indusium ; *ind*², inner indusium ; *ind*² + *am.*, inner indusium and amnion fused ; *am.*, amnion ; *ind*² *c.*, cuticle of the inner indusium ; *ind*² *s.*, granular secretion of the inner indusium ; *am. s.*, amniotic secretion ; *v.*, yolk ; *cl.*, columella ; *gb.*, germ-band.

acceded that the process briefly described in the above paragraph, though now occurring in comparatively few embryos, is very probably the more primitive process, whereas the slurring observed in so many cases is to be attributed to an unquestionably rudimental condition of the organ.

By the time the folds have closed over the indusium the abdomen of the embryo has sunk into the yolk to a considerable extent, presenting in surface view the appearance of Fig. 4. The organ seems to undergo no further change till the embryo has almost left the ventral face of the egg. Then, as we have seen, it begins to increase by spreading. An early stage in this process is shown in section in Fig. 20. No change is perceptible in the serosa, which is now independent of the organ; the outer indusium (*am*^{1}) is much attenuated, as may be seen by comparison with Fig. 19. Its cells have assumed the same shape and dimensions as those of the superjacent serosa; only along the edges of the disk, where the outer becomes continous with the inner indusium, or body of the organ, do the cells still retain their original shapes. In the body of the organ the cells are arranged in two irregular rows, whereas in the previous stage (Fig. 20) there were three. This diminution in the number of cell-rows is the result of horizontal spreading, a process which also accounts for the stretching of the outer indusium as indicated by the flatness of its cells. At *nn* is seen one of the large modified nuclei, which has persisted unusually late.

In Fig. 22 I give a section of the indusium seen in surface view in Fig. 5. The spreading of the cells has progressed till the organ lies like a saddle on the ventral face of the egg, covering nearly half of its circumference. The serosal layer (*s*) is, of course, unaffected. The outer indusium (*am*^{1}) is stretched to such an extent that its cells are united only by an exceedingly thin and in many places, almost imperceptible layer of protoplasm. The inner indusium now consists of a single row of cells, instead of two rows as in the preceding stage. It is in about the same state of tension as the outer layer in Fig. 19.

3. *The Development of the Embryo from the Time of its Reaching the Dorsal Yolk to Revolution.*

In the foregoing paragraphs the development of the embryo was traced to Stage E, when the germ-band hangs festoon-like

in the yolk with its cephalic amnion applied to the ventral serosa and the amnion overlying its terminal abdominal segments applied to the serosa covering the dorsal yolk. No sooner has the caudal end become fixed than the head is detached from the ventral face of the egg and the embryo swings back, straightens out, and becomes applied full length to the dorsal serosa. The movements whereby this condition is attained resemble the movements of a leech in passing from one side of a test-tube to the opposite surface; holding fast to the glass by means of the oral sucker, the tail is stretched out till it reaches the opposite surface, when the anterior end is loosened and the body drawn over. There is, however, a marked difference between the embryo and the leech since the body of the former is not contracted during its transition.

Fig. 5 represents a rather rare condition in that the procephalic lobes lie at the same level and are symmetrically disposed with respect to the long axis of the egg. More frequently the germ-band is twisted during its transition so that one of the procephalic lobes reaches further forward than the other on the surface of the yolk. Sometimes it is the left lobe which extends further forward but more frequently it is the right. The twist in the germ-band occurs in the thoracic or abdominal region, more often in the former, the abdomen being nearly straight. I take this twisting of the embryonic axis to indicate that the germ-band executes a screw-like movement while penetrating the yolk, and I believe it to be perfectly normal, having observed it in the majority of embryos. Traces of this twisting are clearly discernible even in embryos which have almost straightened on the dorsal surface.

As a consequence of the passage of the embryo through the yolk in the manner above described, the germ-band has shifted its position from the median convex ventral to the median concave dorsal surface of the yolk, so that it is now reversed: originally its head pointed to the tapering anterior pole, now it lies with its head directed towards the blunt posterior pole of the egg. The amnion, of course, accompanies and remains in close contact with the ventral surface of the embryo during all this time.

During or more frequently at the close of the embryo's migration the primary serosa secretes from its whole outer surface a thin chitinous cuticle. In my preliminary notes ('90[b], '90[c]) I wrongly designated this cuticle as the vitelline membrane, an error which is, to a certain extent, pardonable, inasmuch as the layer in question is structurally exactly like the vitelline membranes of other insects. But it certainly cannot be homologized with these membranes since it is secreted during a comparatively advanced stage by an embryonic cell-layer, the serosa, and not by the surface protoplasm of the unsegmented egg.

As soon as the embryo has taken up its position on the dorsal surface, the yolk segments; each vitellophag appropriating as many of the yolk-bodies as the radiating filaments of its cytoplasm can hold together and fashion into a rounded mass. Apparently the process is delayed in order that the passage of the embryo through the yolk may be facilitated, for obviously the embryo will move more easily over a prescribed path through a mass of small mobile particles than between large masses formed by the aggregation of such particles. The yolk-masses, at first very distinctly marked, soon fuse with one another so that their boundaries can be traced only by reference to their centres, which coincide with the nuclei of the vitellophags.

After leaving the ventral face of the egg the embryo increases greatly in length. Just before burying its tail in the yolk and while still completely on the ventral surface it measured only .7 mm.; now it measures 1.7 mm. This increase in length, as will be inferred from the foregoing description, is due to two causes: an intercalation of new segments in front of the anal plate to complete the abdomen, and a stretching of the segments thus arising.

A glance at Fig. 6, which represents an embryo in the stage of its greatest elongation on the dorsal surface, shows that many important changes have taken place since it left the ventral surface. The cephalic and thoracic appendages have assumed a more definite character. The labrum (*lb.*) has suddenly appeared, the first and second maxillæ (mx^1, mx^2) have

each become trilobed, while the metathoracic leg (*p*3) already exhibits unmistakable traces of its characteristic thickening in the larva and imago. The pleuropodia (*pl.* (*ap*^{1})) stand out clearly from the edges of the first abdominal segment. Shining through the stretched ectodermal layer of the abdominal segments may be seen the paired mesodermal somites (*coe.*), or mesomeres. The anal plate with its pair of cerci (*cc.* (*ap*^{11})), and the anus are definitely established. A faint neural furrow runs from the mouth to the anus, and in the thoracic region faint metameric indications of the ganglia are apparent. All these important changes have taken place within the yolk during the transition of the embryo. This renders their study on hardened material very difficult, for although the embryo may be dissected away from the yolk, it is so much curved that it can be mounted only in pieces, and the yolk is at this period so difficult to cut that only fragmentary series of sections can be obtained.

One of the most interesting changes undergone while the embryo is still in the yolk is the appearance of the labrum. In Fig. 6 (Stage F) the labrum is a distinctly unpaired circular appendage. But that it has a paired origin I infer from a transverse section, part of which is represented in Fig. 35. This passes just in front of the mouth of an embryo but little older than Stage E. The appendage (*lb.*) is here seen to be distinctly bilobed although it does not yet project beyond the general level of the head. This bilateral condition is speedily slurred over and the organ grows into an unpaired and in most embryos perfectly circular disk overhanging the mouth. Very rarely, as in Fig. 7 it may show traces of its paired origin even during later stages.

Let us return to the indusium which we left as a thin round plate gradually spreading over the yolk just beneath the ventral serosa. The outlines of this plate are not always circular but exhibit traces of lobulation (Fig. 5). The spreading is at first uniform along its whole circumference so that the organ soon assumes the shape of a circular scroll clasping the egg. Its lateral edges approximate on the dorsal surface just over the ventral face of the embryo but are temporarily arrested

in their growth before they unite. The anterior and posterior edges, however, continue to advance without interruption, so that the disk if spread out on a plane surface would in its successive stages represent a series of ellipses with constant short axis but continually increasing longitudinal axis. In this manner the disk grows towards either pole while enveloping the egg laterally. The edges of the organ continue to approximate on the dorsal surface but stop growing just before they meet. Hence, when the egg is viewed from the dorsal surface a long, narrow slit is seen extending nearly its entire length and separating the dorsal edges of the organ. It is not till the anterior and posterior edges have nearly or quite reached their respective poles that this slit closes with the fusion of the edges of the organ. The raphe is at first so weak that the edges may be broken apart by slight pressure with the needles, but it soon becomes permanent and the egg is now completely enveloped by two further membranes — the inner and outer indusia. Before the fusion of these two membranes the amnion of the embryo was in contact with the serosa but now that the edges of the indusia have worked their way in between the serosa and amnion, the latter comes to lie in contact with the inner indusium. Henceforth the serosa is excluded from taking any part in the development of the embryo; both its position and function are now usurped by the inner indusium.

One is enabled to follow the different stages in the progress of the indusium, from its disk-like condition on the ventral yolk to the complete union of its dorsad-growing edges, by means of a peculiar secretion of its inner layer. This is a brownish or blackish granular substance, probably some urate, which appears to be secreted by all the cells of the inner indusium and which gives the organ the appearance of a large brown blotch in a stage a little older than E. At first pale and hardly perceptible, this spot gradually deepens in color till its advancing edges become distinctly outlined on the underlying yolk. A clear idea of the closure of the edges may be obtained from Fig. III, A–C. The dark granular secretion is shown in Fig. 6 at *cnvl.*

Soon after the union of the edges of the outer and inner indusial layers a chitinious cuticle is secreted by the outer surface of the latter. This cuticle is thicker and seems to be of a deeper hue than the cuticle secreted by the serosa. It

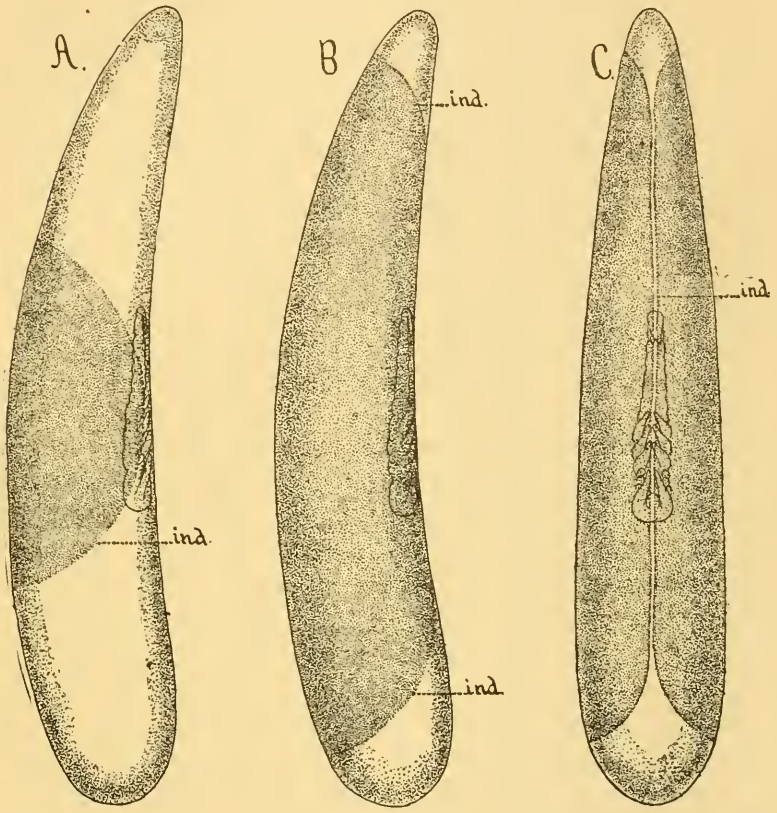

FIG. III.

Two stages in the spreading of the indusium. *A*, lateral view of egg just after the arrival of the embryo on the dorsal yolk ; *B*, lateral view of the egg with the indusium nearly reaching the poles ; *C*, same egg seen from the dorsal surface.

definitely excludes the outer indusium from any further share in the development of the embryo. Even in Stage E, this cell-layer was reduced to an exceedingly thin membrane. (Pl. III, Fig. 22, *am.*[1]) It seems to fuse with the serosa and to retain a connection with the inner indusium only at the ex-

treme anterior pole of the egg. I confess, however, that my observations on this envelope are rather unsatisfactory.

After the completion of the processes described in the preceding paragraphs we may distinguish several envelopes in a median transverse section of the egg. Passing from without inwards we have (1) the chorion, (2) the Blastodermhaut-like cuticle secreted by the serosa, (3) the serosa, (4) the outer indusium, (5) the layer of dark, granular secretion, (6) the cuticle secreted by the inner indusium, (7) the inner indusium and (8) the amnion. While envelopes 1–7 invest the whole egg, layer 8, the amnion, covers only the embryo.

The general development of the embryo has been traced to Stage F, when it lies as a straight and attenuated body on the dorsal yolk with its head directed towards the caudal and its tail towards the cephalic pole of the egg.

Like all other insects that have a stage during which the body is greatly elongated (Coleoptera, Diptera, Lepidoptera) *Xiphidium* passes into a series of stages during which the germ-band is gradually shortened. The shortening is accompanied by a broadening of all the segments, a growth of the appendages, and very important internal changes. The completion of this process is reached in Stage G (Fig. 7). Besides a greater development of the appendages seen in Stage F, Fig. 7 also shows that the abdominal appendages have appeared. Of these there are nine pairs, exclusive of the pleuropodia and cerci, so that in *Xiphidium*, just as in *Blatta* and many other insects, every segment of the abdomen bears a pair of appendages. Starting with the basal segment there are eight pairs of stigmata. These are not all seen in the figure. Just back of each pair of tracheal invaginations appears a second pair of ingrowths — the metastigmatic depressions — seen as small white spots just outside the appendages, near the posterior edges of their respective segments. They are in line (homostichous) with the tracheal invaginations which occupy corresponding positions near the anterior edges of their respective segments.

The ventral flexure of the abdomen constitutes another very important difference between Stages G and F. In *Xiphidium*

this flexure always takes place between the 7th and 8th segments and is brought about during the shortening of the embryo. It is essentially the same flexure which is found in *Blatta* and in Decapod Crustacea.

In Stage G the antennæ have increased to nearly one-third the length of the embryo. The procephalic lobes on which the segmentation of the brain is plainly visible, have developed greatly. The appendages, instead of projecting laterally, as they do in the younger embryo, are folded over the ventral surface of the germ-band. The nerve cord is distinctly marked out. (See abdominal region, Fig. 7.)

It is in this stage, or one but slightly more advanced, that the embryo passes the winter. Cleavage and the succeeding stages up to F are passed within a month after oviposition — during the warm days of August and September. But even should October and November be mild and sunny, development seems to have come to a temporary standstill on reaching Stage G. Among the hundreds of embryos which I collected during three succeeding autumns, I did not find one that had passed far beyond this stage. Nevertheless if kept in a warm, moist atmosphere during winter, a certain number of eggs will continue their development almost to hatching.

Before passing on to later stages in the development I will here give a brief account of some anomalies in the development of the indusium.

4. *Variations in the Development of the Indusium.*

In the preceding pages I have described what I take to be the normal development of the indusium of *Xiphidium*. A considerable number of embryos (about 100), being nearly one half of the total number examined for the stages thus far described, deviated more or less widely in so far as the indusium was concerned from what I consider the normal type of development. Unfortunately I did not discover the organ till it was too late in the season to obtain a large supply of material in the requisite stages, so that the variations here briefly noticed probably represent only a small fraction of those

observable in a large number of eggs. The variations may be tabulated thus : —

1. Variations in size. Normally the indusium is of the same size as one of the procephalic lobes (.2 mm. in diameter) so that the head of the embryo resembles a clover leaf as long as the organ is attached to it. When the chorion is removed the organ may be distinctly seen with the unaided eye as a milk-white spot on the translucent yolk. Occasionally, however, embryos will be found in which it is less than .1 mm. in diameter, and all variations between this and the normal size may be observed.

2. Variations from the typical circular form. These variations are very numerous and may be regarded as belonging to two classes. In one class the indusium is rounded in outline, while in the other it is ragged and more or less irregular. To the first class may be assigned the oval, cordate and multilobulate varieties not infrequently observed; to the second belong a number of irregularly stellate and rhizopod-like forms. In one of my preparations, midway between the two classes, the indusium is evenly rounded anteriorly and ragged posteriorly along that portion of its periphery which has just broken away from the head of the embryo.

3. There is a variation in the time at which the organ is set free from the head. This cannot be proved directly by observation of the organ itself, for it usually does not begin to form the circular fold till after its isolation, but differences in the embryo, especially in the prominence of the segments and appendages, show that the organ remains attached to the head in some cases longer than in others.

4. Variations in the development of the circular fold. These variations, alluded to above, are characterized by a greater or less distinctness in the folds that give rise to the inner and outer layers. All shades in the process may be found between the distinct and comparatively rare method described and figured (Fig. 3), and the more frequent and obscurer method whereby the three layers are formed by a shifting of the individual cells.

5. Variations in number. I have twice observed two indusia

in the same egg. In the first case the embryo itself was in every way normal, and the first indusium of the normal size and shape, and in the usual position. The second, somewhat smaller, though regularly circular organ, was situated in front of the first and a little to the right of the median line. The distance between the two organs was about double the distance between the first organ and the head of the embryo. The outlines of the second or more anterior organ were less definite than those of the first. The amnion and serosa had formed over the embryo, but neither of the indusia showed as yet any tendency to form envelopes. Whether these two organs were derived from the division of one original præoral cluster of cells, or were originally established as two separate centres on the blastoderm, I am unable to decide. The latter method would seem to be the more probable.

The other case is somewhat singular. The first indusium was normal in size and position, but irregularly heptagonal in outline. The second, situated a short distance to the side of the right procephalic lobe, was not more than a third the size of the first organ and quite regularly quadrangular. The embryo itself was normal and covered with the amnion and serosa. The envelopes had also formed over the two organs, which in this case also probably originated from two discrete centres in the blastoderm. The smaller organ had probably never been attached to the head of the embryo.

5. *The Revolution of the Embryo*

During the first warm days of spring the *Xiphidium* embryo resumes its development. This is characterized for some time by a growth of the germ-band in breadth and length and a lengthening of the appendages. The body of the embryo, which in Stages F and G was much narrower than the egg now becomes almost as broad so that its pleural edges embrace the yolk. This increase in size brings the head somewhat nearer the lower pole, and there soon sets in a decided movement of the whole body in this direction. When the head has almost reached the lower pole, the amnion covering the face

of the cephalic end fuses with the overlying inner indusium. A rent appears in this fused portion of the envelopes and through it the head is soon seen protruding. Gradually more of the body is pushed through the orifice, first the mouth parts, then the thoracic legs and finally the abdominal segments, till the whole embryo comes to lie free on the surface of the yolk in the space between the inner indusium and its cuticle. The amnion and inner indusium, which during the evagination of the embryo have remained united at the edges of the rent are folded over the pleural region of the embryo onto the yolk. The two envelopes now form but a single layer enclosing the yolk like a bag. The inner indusium is united to the edges of the amnion and these in turn are united to the pleural edges of the embryo, with the ectoderm of which the amniotic cells are continuous. The small size of the amniotic cells as compared with the huge flattened elements of the inner indusium enables one readily to distinguish the limits of the two envelopes.

During its evagination from the cavity of the amnion the embryo gradually passes around the lower pole of the egg head first and begins to ascend the convex ventral surface. An embryo freed from all its envelopes except the two that take part in revolution is represented in Fig. 8, in the very act of turning the lower pole. The amnion and inner indusium are folded back over the yolk, the former (*am*) characterized by its small rounded nuclei, the latter (*sr.*) by its large flat elements. The line of juncture of the amnion with the body of the embryo is marked by a denser aggregation of nuclei. The ventral flexure still persists on the dorsal surface.

The cavity of the amnion contains a quantity of serum-like liquid, which during the evagination of the embryo is poured into the space separating the inner indusium from its cuticle. This liquid collecting at the lower pole, may function as a lubricant and cushion, and thus facilitate the movements of the germ-band. In hardened specimens it is found as a granular magma enveloping the appendages. It is not shown in Fig. 8.

In many respects the embryo in Stage H has advanced considerably beyond that represented in Fig. 7. In the head, the

eye is distinctly marked out and its cells are arranging themselves to form the ommatidia, as is evident from the regular series of pale dots. The labrum, now considerably enlarged, is spade-shaped in ventral aspect. The antennæ have grown in length, and the saltatory legs (*p*3) are assuming their definitive characters. The large tapering pleuropodia stand out prominently on the first abdominal segment. Near the bases of the legs the thoracic stigmata are distinctly seen. They had made their appearance in Stage G, but for obvious reasons could not be shown in the figure.

The anterior end of the embryo continues to move up the ventral surface of the egg, straightening out as it rises. Finally the flexed terminal segments of the abdomen are again bent back to their original position in line with the rest of the body. Since their flexure these segments (the 8th–11th) have been the only portion of the body provided with a completed dorsal wall (*vide* Fig. 7). After the bending back of the abdominal tip its segments still retain a certain independence and make no attempt to embrace the yolk of the posterior pole as do the segments in front of them. It is for this reason that the abdomen presents a constriction just in front of the eighth segment. This constriction is especially noticeable in profile view.

The turning of the lower pole of the egg seems to take place very rapidly compared with other equally important processes of development, such as the passage of the embryo through the yolk. I infer this from the relative scarcity of embryos in the act of returning to the ventral surface. I have, however, succeeded in finding all the stages in the process of revolution, and feel quite as confident of having correctly interpreted my preparations as if I had studied the living egg.

6. *The Stages Intervening between Revolution and Hatching.*

Fig. 9 represents an embryo that has just straightened out on the ventral surface of the yolk, which the reader may imagine as extending up beyond the head to nearly twice the

length of the embryo and terminating in the pointed anterior pole. A comparison of Figs. 8 and 9 shows that, although the former embryo has completed its revolution, it is nevertheless in an earlier stage so far as the development of its organs is concerned. This is particularly noticeable in the labrum, antennæ and mouth parts, the eyes and the saltatory legs. Hence we may infer that the time for turning the lower pole is subject to considerable variation.

In Fig. 9 it will be observed that many of the abdominal appendages have disappeared. Pairs are, however, retained on the 8th to 11th segments (ap^{8}–cc, (ap^{11})). The pleuropodia are also still present though concealed behind the bases of the metathoracic legs. The disappearance of the appendages on the 2d–8th segments probably has its immediate mechanical cause in the lateral stretching which characterizes these segments in their attempts to embrace the yolk.

The embryo continues its growth as before in two directions —the body constantly lengthening and thus bringing the head nearer the pointed anterior pole, while its lateral walls, enveloping more and more of the yolk, gradually grow towards each other and finally unite in the median dorsal line. The union begins with the 7th abdominal segment, just in front of the segments which have for some time been provided with a dorsal wall, and continues headward. I am not certain as to what becomes of the amnion during this process. Its cells appear to take no part in the formation of the dorsal wall, but very probably degenerate and become supplanted by the cells of the advancing ectoderm. It must be remembered that a hard and fast line cannot be drawn between the amnion and the pleural ectoderm ; the cells of both structures passing into one another by insensible gradations. My reasons for supposing that the amnion proper takes no part in building up the embryo are mainly of a theoretical nature and will be given in the latter part of this paper.

Concerning the fate of the inner indusium there can be little doubt. While the embryo is continually advancing towards the cephalic pole and enclosing more and more of the yolk— this envelope, which, as above stated, is characterized by

huge flat cells and nuclei, is being as gradually restricted to a more and more limited yolk surface. In consequence of this restriction its component cells become broader radially and narrower tangentially. In this stage the envelope functionally corresponds to the "dorsal organ" of other insects. It cannot, however, be thus designated without still further increasing the number of heterogeneous structures included under that unfortunate term, since the "dorsal organ" of other insects is a thickening of an envelope represented in *Xiphidium* by the serosa.

The thickened inner indusium is soon reduced to a cap of cells on the anterior pointed pole of the egg. As the head of the embryo advances to cover more of this pole, the envelope is pushed further forward and finally stripped from the yolk altogether. The anterior cranial walls then close over the pole and thus effectually separate the yolk from the inner indusium. The latter is reduced to a small conical mass, the cells of which soon show unmistakable signs of degeneration.

Soon after the embryo has thus rid itself of its envelopes and has taken into its mesenteron the whole mass of yolk not utilized in the processes of development hitherto undergone, a chitinous cuticle is shed from its entire surface. This may be designated as the first larval cuticle. It appears first on the ventral abdominal surface and spreads thence headward and dorsad. The progress of cuticularization is readily traceable by staining embryos in this stage, for the parts over which the cuticle is formed will not take the color; where it is being deposited the stain takes faintly and where it has not yet appeared, the stain, of course, penetrates easily. Ayers ('84) observed in *Œcanthus* that the secretion of the cuticle began on the ventral surface of the embryo and extended dorsad. This is just what we should expect from the fact that the dorsal hypodermis is ontogenetically a more recent formation than that of the ventral surface.

The first larval cuticle is about 5 μ thick and consists of three layers. The innermost is apparently homogeneous and stains deeply in Orth's lithium carmine while the middle layer remains clear and vitreous. The outer layer is radially striated

and has the distinctly yellow tint of old chitin. Its outer surface is minutely papillate. On the appendages the cuticle is much thinner than it is on the trunk and though it stains it does not show a differentiation into three layers.

Before shedding the first cuticle the hypodermis secretes a second larval skin which persists till after hatching.

In Fig. IV, I have attempted to represent semi-diagrammatically the condition of the envelopes at a time when the eyes begin to acquire pigment. The chorion (*ch.*) is much distended and the egg larger and more resistent to the touch then it was during the autumn. Passing from without inward we first meet with the cuticle secreted by the serosa (*sr. c*). Then follows the serosa itself (*sr.*) to the inner face of which the remains of the outer indusium (*ind.*^{1}) are applied. At the extreme anterior end of the egg both these cellular envelopes appear to be much thickened and pass into a cylindrical pedicel of granular plasma which I shall call the columella (*cl.*). This in turn is continuous with a conical mass of cells (*ind.*^{2}), the remains of the inner indusium which was stripped from the head in a preceding stage. Its cells, as shown in the figure, are in an advanced stage of disintegration. The cytoplasm of the different elements is reduced to a mass of granules and the chromosomes have become agglomerated into little spheres floating in the clear nuclear plasma. The process of degeneration is similar to that which I have described as occurring in the "dorsal organ" of *Blatta*. Between the mass of degenerating cells and the head of the embryo lies a granular coagulum (*am. s*). This I take to be the amniotic serum which is forced up into the anterior pole by the enlarging of the embryo and the consequent decrease in the space between the body walls and the chorion. The columella and the remains of the inner indusium are held together and thus temporarily prevented from complete disintegration by the thick cuticle of the latter. This cuticle still envelops the embryo and extends forward to the anterior pole where it seems to be attached to the inner face of the outer indusium. Passing further inward we next meet with the first larval cuticle (*lv. c*^{1}), which has been shed, and the second larval cuticle (*lv. c*^{2}), which is still in organic

connection with the hypodermis. In a little later stage than the one here described the columella and the conical lump of inner indusial elements have disintegrated, and can no longer be distinguished from the granular amniotic serum.

The changes in the configuration of the embryo since its arrival on the ventral yolk, relate mostly to the appendages. At first the antennæ are of about the same thickness as the

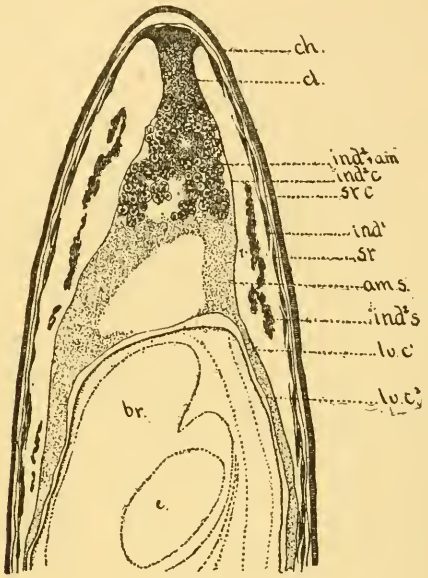

FIG. IV.

Sagittal section through the anterior pole of a *Xiphidium* embryo, with pigmented eyes. *ch.*, chorion; *cl.*, columella; *sr. c.*, Blastodermhaut; *sr.*, serosa; $ind^2 + am.$, remains of the inner indusium and amnion; ind^1, outer indusium; $ind^2 s.$, secretion of the inner indusium; *am. s.*, amniotic secretion; $lv. c^1$, first larval cuticle; $lv. c^2$, second larval cuticle; *br.*, brain; *e.*, eye.

legs. The dark line running parallel with their inner edges, and distinctly marked in Fig. 9, is in section seen to be a mesodermal partition dividing the cavity of the appendage into two tubular sinuses. The antennæ grow directly tailward till their tips reach the femorotibial joint of the hind legs, when they diverge laterally, describe an arc, and then grow forward. When the tips have reached the head further progress is arrested

by the envelopes, but as the growth of the appendages does not cease, the arcs surrounding the hind legs gradually move tailward. This movement is arrested just before the time for hatching, when the antennæ have grown to nearly twice the length of the embryo.

The mouth-parts and thoracic appendages have been gradually assuming their adult characters in the meantime.

The pleuropodia, as described in a former paper ('90[a]), are shed during hatching and just previous to that event may be found attached to the pleural cuticle by means of very slender pedicels.

In the male the appendages of the 9th and 11th abdominal segments persist, the former as the stylets, the latter as the cerci. In the female the cerci also persist but together with them also the pairs on the 8th, 9th and 10th segments (Figs. 9 and 10—*op*[1] (*ap*[8])–*op*3 (*ap*[10]). These are converted into the gonapophyses.

Apart from the eyes little pigment is developed in the hypodermis during embryonic life, unless we regard as such the brown granular secretion of the inner indusium.

A number of eggs kept in the house the greater part of the winter hatched May 15th–18th, but I am inclined to believe that out of doors the regular time for hatching is later, probably not till the end of May. *Xiphidium fasciatum* apparently does not hatch till early in June, since I found larvæ of this species on Naushon Island June 9, which could not have been more than a few days old. Inasmuch as the imagines of *Xiphidium ensiferum* oviposit on the average about Sept. 1st, the whole postembryonic development cannot occupy more than three months. As this Locustid is monogoneutic, nine months is therefore required for embryonic development. Even if we deduct the period of quiescence due to cold weather, it will still be apparent that the embryonic stages must succeed one another very slowly in *Xiphidium* as compared with those of other Ametabola (*e.g. Blatta*), not to mention the Metabola.

7. *The Development of Orchelimum vulgare.*

This Locustid oviposits like many of the smaller members of the family in the pith of dead plants. I found the eggs in Ohio during the last days of September in the stems of the wild lettuce (*Lactuca canadensis*), so common along the edges of fields and thickets, and in the petioles of the common elder (*Sambucus canadensis*). Oviposition probably takes place in the beginning or towards the middle of September. In the case of *Lactuca* and a few other plants which I did not identify, the insects had invariably selected for oviposition the main stem of the flower-panicles. From base to apex this portion of the stem was punctured at intervals, and a single egg thrust into the pith a short distance above each orifice. It is an easy matter to recognize the punctures by the little tufts which the insect evidently gnaws from the woody fibre, before inserting its scimeter-shaped ovipositor. Great care must be taken in splitting the stem, so as not to tear or cut the eggs which adhere very firmly to the pith.

The eggs are larger than those of *Xiphidium ensiferum*, being fully 6.–6.25 mm. long. In shape they are very similar to *Xiphidium* eggs except that the sides are compressed. In the fresh state they are smooth and opaque, and of a pale drab or bluish tint. In this respect, as also in the flattening of their lateral faces, they form a transition to the eggs of our larger Locustidæ, *c. g. Cyrtophyllus concavus*, *Amblycorypha uhlerii* and *Microcentrum retinervis*.[1] The chorion is not readily wetted with water, but like that of the *Xiphidium* egg, immediately becomes transparent when immersed in alcohol. The outer envelope is then seen to have a yellow tint, deepening into brown at the poles.

As would be expected from its close systematic affinity the embryonic development of *Orchelimum* does not differ much from that of *Xiphidium*. I have not seen all the stages, nor have I, as yet, sectioned any of my material, but the stages which I have examined are essentially the same as those

[1] For a description of the eggs of these species see an article on Orthoptera, by Prof. C. V. Riley, in the *Standard Natural History*, Vol. II. pp. 188–189.

described in *Xiphidium*. The embryo of *Orchelimum* passes through the yolk in the same manner as the *Xiphidium* embryo, shortens on the dorsal yolk, then grows apace, moves around the lower pole and finally begins the yolk-enveloping process on the ventral surface of the egg in the same way as the *Xiphidium* embryo. It also develops an indusium which is set free from the head and spreads over the yolk while the embryo is passing through it backwards. In *Orchelimum* the inner indusial layer also secretes a brownish pigment-like substance which enables one to follow its movements as it gradually covers more and more of the yolk. A clear slit is likewise left on the dorsal surface between the folds of the organ. But in the time of closure of this slit *Orchelimum* differs from *Xiphidium*. In the latter insect we found that the slit closed soon after the embryo had straightened on the dorsal yolk, before it had shortened very decidedly. In *Orchelimum* the closure is considerably delayed. The embryo shortens, then grows in length and breadth, passing beyond Stage G of *Xiphidium* and its head nearly reaches the lower pole before the two folds of the indusium meet and fuse. Frequently in this stage, when the embryo is about to revolve, the polar ends of the slit are still open, the membranes having fused over the embryo. In a little later stage, however, the indusium has completely enveloped the yolk.

II. Remarks on Gastrulation in the Orthoptera.

Although many important observations have of late been contributed to the embryology of the Insecta, our knowledge of the formation of the germ-layers in the Orthoptera cannot be said to have made any signal advance. As late as 1889 so few forms of this order had been studied that I felt justified in expressing some doubt as to whether their mesentoderm was formed in the same manner as in the higher Metabola (Coleoptera, Diptera, Lepidoptera). My doubts were confirmed by a study of *Blatta*, when I failed to find the oral formative centre of the entoderm ('89[b]).[1]

[1] We need not go far to seek the reasons for this gap in our comparative studies. The eggs of the Orthoptera are almost without exception extremely

Bruce ('86) appears to have been the first to describe the origin of the mesentoderm from a median ingrowth of the germ-band in the Orthoptera. The species which he studied, is, I have every reason to believe, *Stagmomantis carolina*. His description is very meagre and his figures are unsatisfactory.

More convincing are Graber's figures and description of mesentoderm formation in *Stenobothrus variabilis* ('88, Pl. XIV, Fig. 11; Pl. XV, Fig. 13). His Fig. 11 shows that there is in the median line a distinct infolding of the ventral plate cells — a true invagination. In a more recent paper ('90), the account is briefly repeated without any important additions.

In his recent study of the embryogeny of *Blatta germanica*, Cholodkowsky ('91[a]) gives an account of the formation of the germ-layers more in harmony with what we know of the process in the Coleoptera than the account which I gave. But he has not come to any definite conclusion respecting the formation of the entoderm, and although he maintains that there is a distinct blastoporic groove running the length of the germ-band, he does not figure it in surface view, and most of his sections betray such an amount of distortion in his preparations that one may hesitate to regard the slight depressions in his figures (Figs. 7, 8, 10, etc.) as indicating invagination. Nevertheless I believe from renewed study of the Orthoptera, that Cholodkowsky is correct in deriving the mesoderm from a median proliferation of the primitively one-layered germ-band, and the entoderm from two formative centres — one in the oral and one in the anal region.

In *Xiphidium*, soon after its first appearance, the blastoporic depression, when seen from the surface (Fig. 1), is a straight

refractory from a technical point of view. The cells of the embryo are often smaller and less distinct than they are in the Metabola. Moreover, the great quantity of yolk and its singular brittleness in hardened specimens renders paraffin sectioning most unsatisfactory, and rather than incur the great expenditure of time which working with celloidin involves, the student gladly selects some Coleopteran or Dipteran egg which is all that can be demanded from a purely technical point of view. Nevertheless the Orthoptera constitute, by common consent, one of the most primitive orders of the Insecta; their eggs are large and may be readily procured in great numbers; their development is so gradual that all the requisite stages may be obtained without the least difficulty.

groove extending nearly the entire length of the germ-band and dividing it into two symmetrical halves. Anteriorly the groove is rounded and seems to end rather abruptly, but posteriorly it bifurcates, each of the two grooves thus arising being continued for a short distance to either side till they gradually fade away. There can be no doubt, it seems to me, that the bifurcated termination of the blastopore is the homologue of the similar structure first figured by me in *Doryphora* ('89, Pl. XVIII, Fig. 71; Pl. XIX, Fig. 73) and subsequently seen by Graber ('90) in the corresponding stages of *Lina tremulæ* (Pl. II, Figs. 25 and 27). More recently Cholodkowsky has observed a similar widening of the blastopore in *Blatta*. He attempts to identify it with the posterior depressions of Graber's "lateral gastrulation."

In Stage B (Fig. 2) when the caudal amnio-serosal fold has covered the ligulate portion of the germ-band, the blastopore presents a widening of its anterior end at a point which probably lies just in front of the definitive mouth. This widening was observed in several embryos, and I therefore take it to be a normal occurrence. It also has its homologue in the *Doryphora* embryo (see my Fig. 70, Pl. XVIII, '89). In the stage under consideration (Fig. 2) the anal bifurcation has grown more indistinct and is apparently about to disappear.

The closure of the blastopore proceeds simultaneously in two directions: from its anterior end backwards, and from its posterior end forwards, so that the last portion of the groove to disappear lies in that part of the germ-band which is to become the thoracic or baso-abdominal region.

In sections the groove is seen to be much shallower than it appears in surface view. Along its whole extent its floor is somewhat thickened and in this portion — destined to form the mesentoderm — the cells soon lose their columnar shapes and become more polygonal in outline and more irregular in their arrangement. The groove closes in such a way that no tubular cavity results as in the Coleoptera and Diptera; the cells at the edges of the depression appear to glide over the median elements, so that after the fusion of the edges in the median line the mesentoderm has the form of a solid cord

applied to the inner surface of the germ-band. The process whereby the inner layers are formed is, therefore, a slurred invagination. In this respect *Xiphidium* resembles *Blatta*.

The further differentiation of the mesentoderm is quite as difficult to follow in *Xiphidium* as in other Orthoptera. In these stages the embryo cannot be satisfactorily isolated from the yolk and sectioned by itself, and so friable is the yolk that it is almost impossible to obtain thin sections through the entire egg by the ordinary methods. After studying a few series of sections obtained by means of the celloidin method I can, however, affirm that the invaginated cells give rise to both entoderm and mesoderm. The former has a bipolar origin, as has been made out in the higher forms; in *Apis* by Grassi ('84); in *Hydrophilus* by Heider ('89); in *Doryphora* by myself ('89); in *Musca* by Voeltzkow ('89) and by Graber ('89); and in *Chalicodoma* by Carrière ('90). The anal is considerably larger than the oral formative centre and its elements seem to arise in part from the bifurcation and in part from the deeper portion of the blastopore just in front of the bifurcation.

In *Xiphidium*, just as in the higher Metabola, a pair of entoderm-bands grows towards the baso-abdominal region from either entoderm-pole. Each band, consisting of only one layer of much-flattened cells, meets that of its respective side and then begins to envelop the yolk by proliferation at its ventral and dorsal edges. Transverse sections show that at first the bands are only two or three cells in breadth and that these are closely applied to the dorsal faces of the mesomeres which are formed by this time.

I have made no observations on the relations of the proctodæum to the posterior end of the blastopore, but in regard to the anterior end and its relation to the stomodæum my results are more definite. Figs. 32–34 represent three successive sections through the head of an embryo in Stage D. The last section (Fig. 34) passes through the stomodæum (*st.*) which is just forming as a rounded depression in the cephalic ectoderm. Its large columnar cells are regularly arranged and have their nuclei in the inner ends. The next section (Fig. 33) passes just in front of the stomodæum and cuts two masses of cells in

the median line. The upper of these masses is a thickening of the ectoderm distinctly separated on either side from the elements of the same layer by the peculiar character of its cells. These are much smaller than those of the remaining ectoderm and stain more deeply, especially in the inner portions of the layer. The lower mass of cells is entirely cut off from the ectodermal thickening, though its elements are very similar in size and staining qualities. The ectodermal thickening marks the point where the paired labrum is about to appear (*cf.* Fig. 35). In the next section (Fig. 32), which also passes through the labral region, we again meet with the thickening of the ectoderm. Unlike its portion in the preceding section, it is not bounded below by a curved line, but juts in as a ragged mass of cells, in which it is possible to distinguish a pair of lateral wings and a median projection. The median portion thus proliferated beyond the limits of the ectoderm, is the anterior or oral entoderm centre — the lateral wings I regard as mesodermal. By combining Figs. 32 and 33 the flattened mass of cells underlying the ectoderm in the latter section is seen to be the backward continuation of the mesentoderm. Section Fig. 34 shows that this median unpaired mass splits into two masses, one on either side of the mouth. In this paired condition the bands run backwards through the trunk of the embryo.

Essentially the same condition of the germ-layers in front of the mouth persists till the labrum is definitely formed, as I have observed in a few series of sections. It is difficult to account for the late and intimate union of the mesentoderm with the ectoderm in the labral region, unless we suppose that the blastopore originally extended as far forward as this region and here closed in such a manner that the three layers were not at once separated into ectoderm on the one hand and mesentoderm on the other. It is mainly on this supposition that I take the labral region to coincide with the anterior widening of the blastopore seen in Fig. 2. This widening probably does not coincide with the stomodæum, but lies in front of it, and the definitive mouth is a later formation arising independently from the ectoderm alone.

I would here insert a few observations on gastrulation in *Stagmomantis carolina*, *Gryllus luctuosus*, and *Œcanthus niveus*.

In Fig. 12 the egg of *Stagmomantis* is represented in outline for the purpose of showing the relatively small size of the germ-band which arises as in other forms from a thickening of the blastoderm on the ventral face of the yolk. It is seen to lie somewhat nearer the broad posterior than the pointed anterior pole. It is but slightly longer than broad, and its wider anterior end, which is directed towards the upper pole of the egg, foreshadows the procephalic lobes. Fig. 11 shows that the germ-band of the Mantid, unlike that of *Xiphidium*, is raised above the niveau of the yolk and has its marginal cells sharply separated from the extra-embryonal blastoderm — or serosa — as it is now called. This much flattened layer is, nevertheless, encroaching on the edges of the germ-band to form the amnio-serosal fold (*ams.*). At the anterior edge lies a small cluster of cells (*p. o.*) but little larger than those of the germ-band. I believe that these may represent all that remains of an indusium in *Stagmomantis*.

The narrowly pear-shaped blastopore is very short. Sections show it to be a deep groove, which like the median infolding of other forms (*Doryphora*, *Hydrophilus*, *Musca*, etc.) is deepest posteriorly and grows shallower headward. As I failed to find any of the stages immediately following the one figured, I could not trace out the formation of the germ-layers.

According to Bruce ('86, p. 17), who studied the same species of *Stagmomantis*, "When the union of the folds (of the amnion and serosa) is effected and the embryo is separated from the surface and covered ventrally by the amnion, the under layer is formed, as in *Meloë* and *Thyridopteryx* as an ingrowth from the median line of the embryo." This remark, together with his Figs. XLII–XLIV, Pl. IV, shows that he could not have observed the formation of the layers from a groove and that he must have based his inference on a stage later than the one I have figured.

In *Gryllus luctuosus* the blastopore is more like that of *Xiphidium*. The outline of the egg is shown in Fig. 14. The

germ-band is relatively much larger when compared with the yolk-mass than the germ-band of *Stagmomantis*. It arises on the ventral surface very near the lower pole. That such is the correct position of the embryo may be easily ascertained, since the mother-insect thrusts her eggs into the ground with their long axes perpendicular to the surface. In a glass jar containing a few inches of earth, many eggs were deposited between the surface of the glass and the earth, so that the exact position of the apical pole could be noted, and the egg removed and hardened with this pole constantly in sight. Thus it was possible to determine the exact topographical relations of the embryo to the yolk throughout the important stages of early development.

During gastrulation the germ-band of *Gryllus* (Fig. 13) is more elliptical and somewhat narrower than the germ-band of *Stagmomantis*. Its edges are also distinctly marked off from the blastoderm and here, too, the amnio-serosal fold (*ams.*) arises along the entire periphery. The blastopore (*bl.*) is much narrower than the corresponding depression in *Stagmomantis*. It is deepest posteriorly.

The discovery of an invaginate gastrula in *Gryllus* made it extremely probable that this stage had been overlooked in the other members of the same family which have been studied from an embryological standpoint. Neither Korotneff in his study of *Gryllotalpa* ('85), nor Ayers in his study of *Œcanthus* ('84), succeeded in finding an invagination. I was unable to secure the eggs of any of our native *Gryllotalpæ*, but I collected a great number of *Œcanthus* eggs in Ohio during the last days of September. An examination of these soon convinced me that Ayers had not seen the youngest stages in the development of the germ-band. The youngest germ-band that he figures (Figs. 1–18) lies near the posterior end of the egg with its tail pointing towards the micropylar pole. According to Ayers "A tract of the blastoderm along the median line of the ventral (concave) side, lying nearest the deep or primitively head-end of the egg, becomes thickened into a germinal band, which is the first trace of the *body* of the embryo." But this is *not* the first trace of the body of the embryo, nor does it

arise on the concave face of the egg. The germ-band of *Œcanthus*, like that of *Gryllus*, first makes its appearance as a thickening of the blastoderm on the *convex* surface near the lower pole of the egg. This convex surface is, therefore, the ventral surface and the micropyle marks the "primitively head-end" of the egg as is shown by the fact that the procephaleum is originally directed towards this and not towards the opposite pole, which Ayers incorrectly calls the "primitively head-end." The germ-band, however, soon leaves its position on the convex ventral surface and, moving around the lower pole tail first, comes to lie on the concave dorsal yolk. It is clear that Ayers could not have seen the stages preceding the arrival of the germ-band on the dorsal surface, and it is during these very stages that the blastopore forms and closes.

Before turning the lower pole the germ-band of *Œcanthus* resembles that of *Stagmomantis*. Its anterior is distinctly wider than its posterior end and represents the future procephalic region. A narrow, but distinct groove runs from the oral to the anal end as in the forms we have been considering. At the posterior end the groove bifurcates much as in *Xiphidium*. That this median groove gives rise to the mesentoderm admits of little doubt after what has been said of other Orthoptera. The amnio-serosal fold appears to arise simultaneously along the entire margin of the germ-band as in *Gryllus*.

It follows from the observations here recorded, fragmentary as they are in many respects, together with Graber's observations on *Stenobothrus*, that the Orthoptera can no longer be regarded as *hors de ligne* so far as the formation of their germ-layers is concerned. In all the families of the order, save the Phasmidæ, an invaginate gastrula has been found, and there can be little doubt that the investigator who is so fortunate as to study embryos of this family will find in them essentially the same process of germ-layer formation.

The view is now pretty generally held that in the Insecta both mesoderm and entoderm arise from a median longitudinal furrow — the former layer throughout nearly the entire length, the latter only in the oral and anal regions of the germ-band — and that the vitellophags, or cells left in the yolk at a time when the remaining cleavage products are traveling to the surface to form the blastoderm, take no part whatsoever in the formation of the mesenteron, but degenerate *in situ* and finally undergo dissolution. Discussions of the literature on this subject are to be found in the papers of Heider ('89) and Graber ('89, '90), and so few are the facts accumulated since these résumés were penned that I may dispense with an historical consideration of the insect germ-layers in the present paper.

In the interpretation of the insect gastrula the entoderm has always played an important rôle. The origin of the mesoderm has long been known and has been duly provided for in the various germ-layer hypotheses which have from time to time been advanced. But the true origin of the lining of the mid-gut has been ascertained only within the last few years, so that we cannot expect to find an adequate treatment of this layer in the older theories. Led astray by what had been observed in Crustacea and Arachnida, some writers chose to regard the vitellophags as forming the mesenteron and shaped their theories accordingly (Oscar and Richard Hertwig, '81). But now that it has been shown that the vitellophags take no part in forming the lining of the mid-gut, their morphological position is rendered even more obscure, and we are brought face to face with the question: Are the vitellophags a portion of the entoderm which has been set apart very early in development for the purpose of yolk-liquefaction or are they an entirely new segregation of cells belonging to none of the conventional germ-layers? Those who defend the former alternative maintain that the vitellophags of insects are entodermal in function inasmuch as they digest yolk and closely resemble the amœboid Crustacean yolk-cells which are actually converted into the lining of the mesenteron. On the other hand it is urged, that as the yolk-cells arise and function before the blastoderm is com-

pleted and hence some time before the germ-layers are formed, they cannot properly be assigned to the entoderm.[1]

It is probably best to await the results of further investigation before deciding on the phylogenetic relations of the vitellophags to the entoderm. Heider ('89) has also expressed himself to this effect and I fully endorse his opinion when he says: "Immerhin wird man vorläufig über vage Vermuthungen nach dieser Richtung nicht hinauskommen, und ist die Frage nach der Auffassung der Dotterzellen bei dem Nachweise, dass sie an dem Aufbau des Embryos keinen Antheil nehmen, meiner Ansicht nach von geringerer Wichtigkeit."

[1] Besides these vitellophags which with Cholodkowsky ('91[a]) we may call the primary yolk-cells — there are other cells which detach themselves from the blastoderm or embryo and enter the yolk. These Cholodkowsky calls secondary yolk-cells. While the origin of the primary yolk-cells has been quite satisfactorily demonstrated, this cannot be said of those of the second class. They appear to descend into the yolk at different times in different species. Thus, according to Patten ('84), all the cleavage products in *Neophylax* ascend to the surface, the yolk-cells subsequently descending from the blastoderm. I claimed a similar total migration of the cleavage products to the surface in *Blatta* ('89); Cholodkowsky, however, claims that some of the cells never reach the surface, but remain in the yolk. Be this as it may, in later stages I believe it can be shown that cells do migrate into the yolk from the embryo and especially from the entoderm-centres. This was shown by me to be the case in *Doryphora*, where many cells pass into the yolk from either entoderm pole (Pl. XIX, Fig. 82; Pl. XX, Fig. 88). I have since observed an exactly similar phenomenon in *Telea polyphemus* in a corresponding stage of development. Graber, ('89, p. 11) too, has made a similar observation on *Melolontha*, where he saw "vom invaginirten Blastodermwulst aus unter lebhaften Theilungserscheinungen ganze Ströme von Zellen in den Dotter hineinwandern, Zellen die freilich von den primären, gleichzeitig vorkommenden und auffallend grosskernigen Centroblastelementen ganz enorm verschieden sind, und die sich überhaupt durch ihre ganze Beschaffenheit als unzweideutige Abkömmlinge, man könnte sagen Auswürflinge eines wahren Keimblattes, erweisen." So far as the migrant cells described in *Doryphora* are concerned, I am sure they come from the entoderm. They occur only at or near the entodermal Anlagen and may be traced from this germ-layer into the yolk. These cells are not actively dividing like those described by Graber, but actively disintegrating. (May not Graber have mistaken disintegration-figures for caryokinetic figures?) In somewhat later stages no traces of these migrant cells are to be found. The yolk is segmented at the time of their leaving the entoderm and their invasion appears not to disturb in the least the activities of the vitellophags. Whether there is any relation between these evanescent entoderm cells and the "secondary mesoderm" of Reichenbach ('86), the "spores" of F. H. Herrick ('86), or the "chromatin nebulæ" of Bumpus ('91) is a question which cannot be answered at present.

Among those who take a decided stand on the relations of the vitellophags to the definitive entoderm, Graber and Cholodkowsky may be mentioned. Graber ('**89**, p. 10), after introducing the superfluous and inapplicable term "centroblast,"[1] says: Dabei nehme ich zugleich, was indessen kaum misbilligt werden dürfte, stillschweigend auch an, dass dieses gegenwärtig, wie es scheint, von der Darm- and Gewebsbildung ausgeschlossene Zellenlager auch früher niemals eine dem echten Entoderm anderer Thiere entsprechende Rolle inne gehabt habe, sondern vielmehr dem letzteren gegenüber ein neues, wahrscheinlich mit der stärkeren Entwicklung des Dotters im Zusammenhang stehendes Differenzirungsproduct ist."

Cholodkowsky ('**91**[a]) does not dismiss the matter so briefly. Like Graber he draws a hard and fast line between primary and secondary yolk-cells, and admits no phylogenetic continuity between the vitellophags and the definitive entoderm. The vitellophags belong to none of the germ-layers. His reasons for not regarding them as a precociously segregated portion of the entoderm are neither new nor conclusive. Like other recent investigators he admits that the vitellophags are in part digested or discharged from the alimentary tract along with the remains of the yolk after hatching. But he is not satisfied that the yolk-cells should play a humble rôle in the insect economy. Some of them were predestined to a higher function than yolk-liquefaction — viz: to give rise to the blood, the fat-body and even to the germ-cells. He therefore supposes that the vitellophags are undifferentiated cells. But this supposition is not supported by the facts. That they are on the contrary, considerably specialized is shown by their limited function and mobility, their gradual and prolonged growth (especially in some Orthoptera), their inability to undergo caryokinesis or even akinesis, and their suspicious relations to the bacteria-like corpuscles of Blochmann. On *a priori* grounds we should not expect to derive whole sets of tissues from such specialized elements.

[1] Superfluous because we have enough names for these cells already, inapplicable because the termination "blast" is properly applied only to cells or tissues of a germinal character — not to decrepit elements like the yolk-cells.

But a more weighty objection may be adduced. It has been shown by Heider ('89) and Heymons ('90), not to mention many previous investigators, that the fat-body and sexual-cells arise from the mesoderm, and my own studies fully confirm this view. Concerning the origin of the blood there is some doubt, but it should be stated that Cholodkowsky has made no satisfactory observations of his own on this point and that, although some facts point to a derivation of the blood from the yolk-cells, others as definitely point to its origin in the mesodermal layer.

After taking for granted that the vitellophags are undifferentiated cells, that they have nothing and, what is more, never have had anything to do with the entoderm, and that they give rise to blood-corpuscles, adipose-tissue and germ-cells, Cholodkowsky ushers in the parablast theory. It was to have been hoped that this theory might have been permitted to end its days in peace within the confines of vertebrate embryology where it originated. Fortunately, however, it has grown too old and decrepit, even under the skillful medical treatment which it has received from time to time, to be of any service in invertebrate morphology.

There is something almost ludicrous in Cholodkowsky's application of the parablast theory to the Insecta when we consider the methods which he employed in preparing the yolk of the *Blatta* egg. The capsules opened at both ends were subjected to the action of undiluted Perenyi's fluid for 12 hours and the eggs after treatment with the customary grades of alcohol, cleared in green cedar oil 24 hours. Thence they were transferred to paraffine (55–60° C.) and left 3–5 hours. The result of this heroic method is apparent enough in the distortion of the tissues, but its effect on the yolk is quite remarkable.

Both Blochmann ('87) and myself ('89) described the yolk of the *Blatta* egg as consisting of a mass of homogeneous and granular albuminoid bodies sharply polygonal from mutual pressure and interspersed with spherical oil-globules. We also described a peculiar distribution of the polygonal bodies; those of a homogeneous nature constituting an oval central core invested with the granular bodies. I further claimed that the

Blatta egg exhibited a yolk-segmentation which though faint and appearing late was, nevertheless, comparable to the yolk-segmentation in such forms as *Doryphora*.

Cholodkowsky ('91[a]) thus describes the yolk: "So kann ich, z. B., nicht bestätigen, dass der Dotter aus einzelnen polygonalen Dotterkörpern bestehe, wie derselbe von Blochmann (und Wheeler) beschrieben und abgebildet wird. Der ganze Dotter besteht aus einer continuirlichen plasmatischen Substanz, deren Vacuolen grössere und kleinere Fetttropfen enthalten. Die Continuirlichkeit der Dottermasse tritt nun um so deutlicher hervor, je besser die Objekte conservirt sind. Das Bild (ich möchte sagen, das Trugbild) der polygonalen Dotterkörper entsteht durch Bersten des Dotters nach der Bearbeitung mit nicht ganz passenden Reactiven." . . . . "Auch kann ich die Blochmann'sche Unterscheidung des 'inneren' und 'äusseren' Dotters nicht annehmen; der ganze Unterschied in den Färbungsverhältnissen der beiden angeblichen Theile des Dotters lässt sich einfach dadurch erklären, dass die Farbe aus den peripherischen Theilen des Eies leichter als aus den inneren mit Säure ausgezogen wird." And at p. 58 he remarks: "Es ist bemerkenswerth, dass bei *Blatta germanica* eine Dotterzerklüftung vollkommen fehlt. Ich kann also mit Wheeler nicht übereinstimmen, wenn er sagt (p. 359), dass bei *Blatta* der Dotter, wenn auch sehr spät (nach Bildung der Extremitäten) sich furchen soll; höchst wahrscheinlich war Wheeler zu dieser irrigen Annahme durch die ausserordentliche Brüchigkeit des Dotters verleitet."

On reading these criticisms I re-examined my preparations and must emphatically re-assert what I claimed in my description of the yolk of the *Blatta* egg. Among my preparations I find several mature ovarian eggs hardened in Perenyi's fluid — not, however, treated with that vigorous reagent for 12 consecutive hours — and these show the yolk-bodies very distinctly as polygonal masses. There are no traces of a "Bersten des Dotters." Eggs killed in ordinary alcohol and mounted *in toto* show the polygonal yolk-bodies distinctly and in these same specimens the distribution of the different yolk-elements may be followed by carefully focusing. That Cholodkowsky should

be unable to detect the outlines of the segments in the yolk of eggs treated for half a day with Perenyi's fluid is not surprising, especially as this segmentation is of very short duration in *Blatta* as in other Orthoptera. It is present, however, as I have convinced myself from eggs mounted *in toto* and from sections.

If prolonged immersion in Perenyi's fluid can bring about a complete fusion of the yolk-bodies and an obliteration of their true structure, what must be its effect on the vitellophags scattered through the yolk? And how much importance are we to attach to Cholodkowsky's assertion that the fat-body, blood-corpuscles and sexual-cells arise from the vitellophags, and to the parablast theory as applied to the *Blatta*-ovum?

Let us return from this digression to the germ-layers. The curious fact that the definitive entoderm of the Insecta arises from two separate centres — one oral and the other anal — is too recent to have given rise to much speculation. Since the entoderm of other animals arises from a single centre it is tacitly assumed that such must originally have been the case with the Insecta, and that the present bipolar condition must be due to secondary modification. Starting with this postulate, there are, of course, many ways in which the bipartition of the original unipolar entoderm may be supposed to have taken place. Two of these possibilities are worked out in the hypotheses of Kowalevsky ('86) and Cholodkowsky ('91[a]).

Kowalevsky has expressed his views so clearly and concisely that I cannot do better than quote his own words: "Wenn wir jetzt versuchen, diese Bildung des Ento- und Mesoderms bei den Musciden mit der Bildung dieser Blätter bei anderen Thieren zu vergleichen, so sehen wir erstens, dass hier auch eine Art sehr in die Länge ausgezogener Gastrula entsteht, und dass aus dem eingestülpten Teil das Ento- und Mesoderm sich bildet. Also in diesen allgemeinen Zügen finden wir eine Uebereinstimmung. Es scheint mir aber, dass die Parallele noch weiter gezogen werden kann. Namentlich wenn wir der Bildung des Ento-Mesoderms bei *Sagitta* uns erinnern, so finden wir bei derselben dass der eingestülpte Teil des Blastoderms in drei parallele Säcke zerfällt, von

denen der mittlere das Entoderm liefert, die seitlichen aber das Mesoderm. Bei den Musciden entsteht auch eine solche Einstülpung wie bei *Sagitta*, und auch der mittlere Teil — allerdings nur an beiden Enden vorhanden — liefert das Entoderm, die seitlichen Teile liefern das Mesoderm: also ähnlich dem, was wir bei der *Sagitta* beobachten. Um die Aehnlichkeit weiter zu führen, kann vorausgesetzt werden, dass bei der so in die Länge gezogenen Gastrula der Insekten der mittlere, das Entoderm liefernde Sack so ausgezogen ist, dass er in der Mitte ganz verschwindet und nur an seinem vorderen und hinteren Ende bestehen bleibt. Bei dieser Auffassung wird es von selbst schon folgen, dass die sich schliessende Rinne fast auf ihrer ganzen Länge nur das Mesoderm liefert.

Jetzt bleibt noch die Frage übrig: wie verhalten sich die Flächen der Gastrula zu den Flächen des sich bildenden Entoderms. Bei der *Sagitta* wird die äussere Oberfläche der Blastula nach der Einstülpung zur inneren Oberfläche des Darmkanals, d. h. die Seiten der Zellen, welche bei der Blastula nach aussen gerichtet waren, werden im Darmkanal nach seinem Lumen gerichtet. Bei den Insekten kann dasselbe auch vorausgesetzt werden. Wenn wir uns die eingestülpte Rinne vorstellen, so sind deren Oberflächen ganz ähnlich gelagert wie bei der Gastrula; wenn wir weiter die Bildung der beiden Entodermanlagen dem mittleren Sacke der *Sagitta* vergleichen, so bleibt die Lagerung der Zellenflächen noch ganz dieselbe. Wenn wir dann voraussetzen, dass der mittlere Sack durch die weite Ausbreitung und durch das Eindringen der Masse des Dotters gewissermassen in seinen vordern und hintern Teil zersprengt ist, so kommt der Dotter ins innere des hypothetischen Sackes, und die Zellen, die den Dotter bedecken, werden zu dem Dotter in derselben Beziehung stehen, wie bei der *Sagitta* zu der eingestülpten Fläche."

A few years after these remarks were written Heider ('89) and myself ('89) at about the same time published observations on the Coleopteran germ-layers which seemed to support the hypothesis of the celebrated Russian embryologist. As further support to Kowalevsky's view I believe we may point to such gastrulas as that of *Stagmomantis* described above. In this

gastrula, which is so very short and broad, we may suppose that the oral and anal entoderm-centres are really continuous, covering the floor of the blastopore from end to end. In sections, it is true, I failed to detect any differentiation of the cells forming the walls of the furrow, into entodermal and mesodermal elements, but this would also be the case in the elongated gastrulas of other insects in a correspondingly early stage (*Xiphidium*, *Doryphora*). As favoring a purely mechanical separation of an originally single entoderm Anlage, it may be noted that the most rapid elongation of the germ-band occurs at a time when the entoderm is differentiating from the mesentoderm Anlage. There is probably more than an accidental correlation of these two processes. During this time some germ-bands (*Doryphora*, *Xiphidium*) double their length.[1] Inasmuch as the lengthening of the superficial layers of the embryo is much more rapid than the differentiation of the entoderm, this germ-layer must lag behind. In most insects the embryo is at the time of its greatest elongation much longer than the yolk-mass and must again shorten to the length of this mass, so that a rapid proliferation of the entoderm may be superfluous, since this layer would have to readjust itself to the yolk with the contraction of the embryo. It may, therefore, be an advantage for the entoderm to be somewhat retarded in its growth. In the Orthoptera, where the embryo lengthens rapidly, shortens, and then lengthens again to envelope the yolk we may suppose, for reasons to be given in the sequel, that yolk has been acquired. This seems also to be suggested by the histological structure of the embryonic entoderm; this layer consisting of large polygonal cells in the Coleoptera, which have only a medium amount of yolk, while in the Orthoptera the attenuate entoderm-bands consist of a very few flat cells.[2]

It is probable that when more forms have been carefully

[1] *Blatta* forms a very rare exception in this respect.

[2] A very similar condition may be observed in the case of the blastoderm. In the Coleoptera and Diptera, which have a medium or small amount of yolk, the newly formed blastoderm is a deeply columnar epithelium ; in the Orthoptera it is a true pavement epithelium.

studied a method of entoderm formation midway between the unipolar and bipolar methods will be found to obtain in some insects. We must admit that a contribution of elements to the entoderm from the interpolar region of the furrow is not with certainty precluded in several of the species which have been studied. Thus Heider, ('89) while inclined to believe that such a contribution does not take place in the anterior portion of the germ-band, believes that it may take place in the posterior abdominal segments. Cholodkowsky ('91[a]) is inclined to accept a still more diffuse origin for the entoderm. "Untersuchungen zahlreicher Schnittserien machen es wahrscheinlich, dass an verschiedenen Stellen des Keimstreifens sich einzelne Zellen vom äusseren Blatte abspalten und an der Bildung des inneren Blattes betheiligen, so dass die Entstehungsart des letzteren sehr complicirt erscheint."

Starting with the same postulate as Kowalevsky, viz: that the bipolar is derivable from a unipolar condition of the entoderm — Cholodkowsky ('91[a], '91[b]) proceeds to account for this phenomenon in a very different way from his compatriot. He takes the small, round blastopore of *Astacus*, stretches it till it equals the insect blastopore in length, introduces a number of modifications — such as the median groove and the pairs of lateral depressions and believes that he has found an explanation "sehr klar und ungezwungen" for all the different blastopores, not only in the Insecta, but also in the meroblastic eggs of vertebrates. It was Kleinenberg who said: "Gewagte Hypothesen, kühne Schlüsse nützen der Wissenschaft fast immer, die Schemata schaden ihr, wenn sie die vorhandenen Kenntnisse in eine leere und dazu noch schiefe Form bringen und beanspruchen tiefere Einsicht zu geben." The latter part of this aphorism seems to be particularly applicable to Cholodkowsky's exposition. As Graber ('91) has briefly pointed out, there are no grounds for comparing the *Astacus* blastopore with the entire insect blastopore. In the Decapods this orifice is confined to the anal region and if comparable at all to the median furrow in insects, must be compared with the caudal entoderm pole. This is all that is admissible, since the mesoderm of the Decapoda arises from the anterior lip of the blastopore and

proliferates headward. That such is its origin has been shown by Bobretzky and Reichenbach for *Astacus* ('86), by Paul Mayer for *Eupagurus* ('77), and by Bumpus for *Homarus* ('91). To Cholodkowsky both the extent and position of the blastopore are of little consequence as is abundantly evident from his reply to Graber's well-founded objection. It is this very neglect of what are generally and, I believe, rightly considered two of the most important matters in the discussion of the germ-layers, which stamps Cholodkowsky's hypothesis as superficial and inadequate.

There is, however, one redeeming suggestion in his hypothesis, viz: that the diverging grooves at the posterior end of the blastopore in insects may correspond to the "Sichelrinne" of vertebrates. Certainly the relations of the grooves to the median furrow in *Xiphidium* (see Fig. 1.) closely resemble in surface view the relations of the "Sichelrinne" to the primitive streak in the chick as figured by Koller ('81) and in the triton as figured by Oscar Hertwig ('90, p. 99).

While most investigators probably agree with Kowalevsky and Cholodkowsky in deriving the bipolar from a unipolar condition of the entoderm, Patten does not share this view ('90). In his opinion, which is based on Kleinenberg's interpretation of the gastrula, the blastopore is restricted to the oral region, and such depressions as occur at the posterior end of the germ-band, as well as the formation of teloblasts in that region, are supposed by him to have no connection with the blastopore, but to be merely the instruments of unipolar growth. "The Arthropod body represents an outgrowth from the trochosphere, but the trochosphere itself, the coelenterate stage, has disappeared. Hence there is no such thing as a gastrula in Arthropods and strictly speaking, no germ-layers." It is clear that this view must stand or fall with Kleinenberg's theoretical conclusions on which it is based, and we may venture to say that E. B. Wilson's recent work ('90) has rendered this foundation very insecure, notwithstanding Patten's rather confidant assertion that "in *Lopadorhynchus* it is certain that the greater part of the mesoderm arises from the ectoderm at the growing tip of the tail, and has nothing to do with primitive mesoderm."

But it would be out of place to consider the widest bearings of Patten's hypothesis in this paper since I am concerned with it only in so far as it bears on the germ-layers of insects. Starting with the assumption that the blastopore is confined to the mouth, he attempts to show that the median furrow is a purely secondary structure. "That the median furrow of insects is merely an ontogenetic adaptation is sufficiently evident from the fact that it may be present or absent in closely related forms." This, however, is not the case. On the contrary the furrow or a slight modification of it is, we have every reason to suppose, universally present in the Insecta, at least in the Pterygota, and this wide occurrence of the structure is one of the surest indications of its high antiquity and phylogenetic importance.

In the latter part of his discussion Patten admits that there are "structures in Arthropods which may represent remnants of gastrulas. For example, if the mouth and œsophagus of Arthropods is primitive — and there is no reason to suppose it is secondarily acquired — then we must look for primitive entoderm at its inner end. I have figured in 'Eyes of Acilius,' at the very anterior end of the embryo, a great sac of entoderm cells which probably arise by invagination, although the process was not directly observed. The sac, which soon opens outward by the œsophagus, afterwards becomes solid, and finally is converted into two longitudinal bands, one on either side, extending backwards to the middle of the body, where they become continuous with similar bands extending forwards from the posterior end of the embryo." Patten admits that true entoderm is formed at two widely separated regions of the body, but he implies that only the anterior centre is comparable to the entoderm of other animals, the posterior centre being a new and purely adaptive formation. It is just here that his theory appears to me to fail, since it does not explain why the oral and anal centres should resemble each other so very closely in origin, method of growth and histological structure.

III. The Indusium and its Homologues.

In none of the Pterygota hitherto studied has there been found any trace of a structure comparable to the indusium of *Xiphidium* and *Orchelimum*. The organ appears to have been retained by the Locustidæ and completely lost by the embryos of other winged insects. In some of the Apterygota, however, there is an embryonic organ which gives a clue to the possible homologues of the indusium. I allude to the so-called "micropyle" of the Poduridæ.

During the summer of '91 I was so fortunate as to secure the eggs of *Anurida maritima* in great numbers. They are much larger than any of the Poduran eggs hitherto studied — so large that they may be removed from their choria by means of dissecting-needles and partially stained for surface views. It is also an easy matter to obtain good sections.[1] When first deposited the eggs are provided with a thin transparent chorion and vitelline membrane, but after cleavage, which is total, is completed and the blastoderm formed, a yellow, peculiarly striated chitinous membrane is secreted from the surface. The egg then enlarges till the chorion and vitelline membrane are burst. The striated membrane was described by Ryder ('86), but he failed to observe that it is attached to a large circular ring — the "micropyle." In section (Fig. V) this organ is seen to be a very decided thickening of the blastoderm which at this time covers the whole yolk-mass as a single layer of minute columnar cells. In the "micropyle" the cells and nuclei are much enlarged and often considerably vacuolated. Surface views prepared according to the partial staining method show that the embryo is already faintly outlined on the yolk and that the ring-shaped organ lies just in front of its head (Fig. VI). The egg being spherical, the embryo is curled in a semicircle and the "micropyle" thus comes to lie on the dorsal surface nearer the head than the tail of the germ-band. In the figure a more advanced embryo is represented as spread out on a flat

[1] I mention this because the few fragmentary accounts that have been published on the development of the Poduridæ are based on the study of the embryo viewed through the chorion and other envelopes. This has given rise to some errors which I hope to point out in a future paper.

surface. The resemblance of the "micropyle" to the indusium is apparent at a glance (*cf.* Fig. 2, Pl. I). I have followed the organ in *Anurida* through the later stages by means of sections and find that it persists for some time as a simple thickening of the blastoderm, still connected with the peculiar striated membrane which stands away from the surface of the blastoderm at all other points. Finally, when the embryo has become flexed dorsoventrally and the body-walls are closed, it sinks into the yolk and is absorbed.

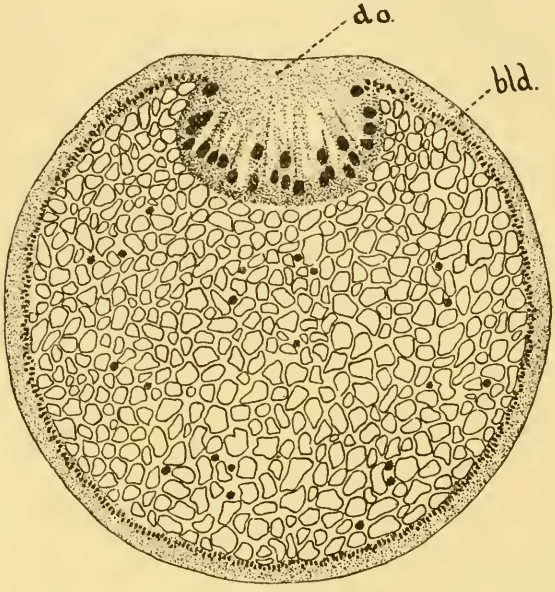

FIG. V.

Median section of the egg of *Anurida maritima*. *d.o.*, "micropyle"; *bld.*, blastoderm.

Although much simpler in its structure, I do not hesitate to homologize this "micropylar" organ in *Anurida* and the Poduridæ in general with the indusium of *Xiphidium*. A possible objection to this homology, on the ground that the indusium arises on the ventral face of the egg, while the Podurid "micropyle" is dorsal, has little weight, since the organ bears in either case the same relation to the head of the embryo. Provided, therefore, the egg of *Anurida* were to acquire yolk

and become greatly elongated, like the *Xiphidium* egg, the micropylar organ must come to lie on the same surface of the yolk as the germ-band.

It has been repeatedly suggested, and, I believe, on very good grounds, that the Podurid "micropyle" is the homologue of the crustacean "dorsal organ." In both groups the organ arises soon after the germ-band is mapped out on the yolk, and in both groups it is a circular or oval thickening of the blastoderm lying in the median dorsal line distinctly nearer the head than the telson. In the Crustacea its centre often shows a depression to the walls of which the Blastoderm-haut is attached, while standing away from the surface of the egg at other points. An exactly similar condition obtains in the Poduridæ; a slight depression marks the centre of the organ in *Anurida*, while in *Anurophorus* (Lemoine, '87) there appears to be a deep pit at the attachment of the chitinous envelope. This depression is comparable to the depression seen in Fig. 3 in *Xiphidium*, where the circular fold is encroaching on the disk.

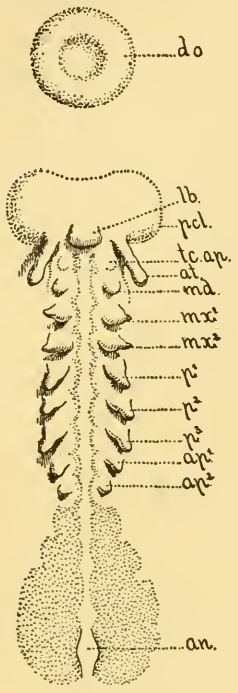

FIG. VI.

Embryo of *Anurida maritima* spread out on a flat surface. *d.o.*, "micropyle" *lb.*, labrum; *pcl.*, procephalic lobe; *at*, antenna; *tc. ap.*, minute appendage of the tritocerebral segment; *md.*, mandible; mx^1, mx^2, first and second maxillæ; p^1–p^3, first to third thoracic appendages; ap^1, first abdominal appendage (= left half of collophore); ap^2, second abdominal appendage; *an.*, anus.

Up to the stage represented in the figure just referred to, the indusium will bear close comparison with the crustacean "dorsal organ." In the first stages of its spreading it also resembles to some extent the saddle-shaped "dorsal organ" of *Oniscus*, *Porcellio*, and *Ligia*. But beyond this point it differs widely from its homologues, and it is difficult to see why it

should persist, and instead of sinking into the yolk, envelop the whole egg, secrete a granular and thereupon a chitinous layer, and finally, during revolution take on the function of a true serosa. That the organ is rudimental is shown by its tendency to vary, especially during the earlier stages of its development; that it still performs some function is indicated by its somewhat complicated later development and by its survival in but very few forms out of the vast group of Pterygotous insects. This seeming paradox may be explained, if we suppose that the indusium was on the verge of disappearing, being the last rudiment of some very ancient structure. As such a rudiment it no longer fell under the influence of natural selection, and for this reason began to vary considerably like other rudimental organs. Some of these fortuitous variations may have come to be advantageous to the embryo, and were perhaps again seized upon by natural selection; the nearly extinct organ being thus resuscitated and again forced to take an active part in the processes of development.

Pursuing the homologies of the indusium still further we come to the Arachnida, where we find in the primitive cumulus of spiders a structure comparable in many ways to the Podurid "micropyle," as v. Kennel ('85, '88) and Lemoine ('87) have suggested. There is, however, so much difference of opinion regarding the position and signification of the primitive cumulus that I should hardly be willing to agree with these authors, were it not for two of Claparède's figures of the *Pholcus* embryo ('62, Figs. 6 and 7, Pl. I). These show in the median dorsal line a thickening which forcibly recalls the "micropyle" of *Anurida*. Still it must be admitted that Claparède has failed to prove the identity of this thickening with the primitive cumulus.

In Pentastomids the "facette" or "cervical cross" described by Leuckart ('60) and Stiles ('91) is very probably the homologue of the crustacean dorsal organ and the insect indusium.

Although no homologous structure has yet been detected in the Myriopoda, the occurrence of a dorsal-organ-like structure in such widely separated groups as the Hexapoda, Araneina, Pentastomidæ, and Crustacea is sufficient reason for regarding

it as exceedingly ancient and as well-developed before the existing subdivisions of the Arthropoda were established. To seek a homologue of the "dorsal organ" among existing annelids may be regarded by some as a hopeless undertaking. Still I would call attention to Apathy's observation ('88) on *Clepsine bioculata*. The adult of this species has long been known to possess a chitinous plate in the median line between the head and the præclitellum. Apathy finds that this plate is the remnant of an embryonic sucking-disk, the glandular cells of which secrete a bundle of byssus-like threads that harden on contact with the water and serve to anchor the undeveloped young to the ventral concavity of the mother-leech. A similar organ is also found in the young of *Clepsine heteroclita*. It is certainly no great step from this embryonic sucking-disk of the Hirudinea to the Phyllopod "cervical gland" which is also used as a sucker, and which Fritz Müller ('64) and Grobben ('79) regard as homologous with the "dorsal organ" of the Amphipoda.

IV. The Envelopes and Revolution of the Insect Embryo.

1. *The Amnion and Serosa.*

The formation of two cellular envelopes, the amnion and serosa, by a folding of the primitive extra-embryonal blastoderm, is rightly considered one of the most characteristic features of the Hexapod embryo. The envelopes are not, however, common to all insects. An amnion is completely lacking in the Poduridæ,[1] and consequently the extra-embryonal blastoderm in these forms is strictly comparable to the corresponding portion of the blastoderm in Crustacea, Myriopoda, and Arachnida. This is proved by the fact that it ultimately forms the definitive dorsal body-wall. So far as our present knowledge extends, the Apterygota may be regarded as Hexapoda Anamniota, and

[1] Lemoine ('87) describes a cellular "membrane amniotique" in *Anurophorus*, but he does not represent it in his figures and did not study it in section. I therefore incline to doubt the correctness of his observation, especially as I can find no traces of a cellular envelope in the *Anurida* egg, which on account of its size is a far more favorable egg for study than that of *Anurophorus*.

placed over against the Pterygota, which are characterized by the possession of an amnion (Hexapoda Amniota). There is a gap between these two groups of insects similar to the gap between the amniote and anamniote vertebrates. Whether it will be filled by the future study of such orthopteroid forms as *Machilis*, *Lepisma* and *Forficula* remains to be seen. For the present I am inclined to believe that the amnion first made its appearance in the ancestral Pterygota. Even if it be contended that the amnion was once present in the Apterygota and subsequently lost, its origin could not consistently be pushed further back than the Hexapoda, since this envelope is lacking in the Myriopoda, which, there is reason to believe, lie in the direct line of descent. The proof that the so-called amnions of *Peripatus*, Scorpions and Pseudoscorpions are the homologues of the insect amnion is not forthcoming. Judging from the few descriptions of their formation, they appear to have arisen independently within their respective groups.

Just as many of the Pterygota develop only rudiments of wings or have altogether ceased to develop these organs in the adult state, so the embryos of the Pterygota in some cases develop only rudimental envelopes or none at all. It is reported that the amnion is lacking in the Proctotrupid Hymenoptera (Ayers, '84) and rudimental in Muscidæ (Kowalevsky, '86; Graber, '89) and viviparous Cecidomyidæ (Metschnikoff, '66). Certain ants of Madeira are incidentally mentioned by Metschnikoff as having the envelopes represented only by a small mass of cells in the dorsal region. The absence or abortion of the amnion is almost certainly a secondary condition. The Proctotrupidæ are egg-parasites and undergo an extremely aberrant embryonic and larval development. Both these and the other insects mentioned belong to groups characterized by high specialization. This is notably the case with the ants and with the Muscidæ which show considerable aberration in their embryonic and larval stages. The pædogenesis of the Cecidomyids studied by Metschnikoff stamps them also as aberrant. Moreover the embryos of other Orthorrhaphous Diptera (Simulidæ, Chironomidæ, Tabanidæ) have perfectly normal envelopes.

Many attempts have been made to explain the origin of the amnion in insects. It first appears abruptly and fully developed in the Orthoptera just as the vertebrate amnion appears abruptly in the Reptilia. One school, represented by Nusbaum ('87) and v. Kennel ('85, '88), regards the insect amnion as a structure of high phylogenetic value and would trace it to some organ in the lower Arthropods or in the worms. According to another view advocated by Will ('88) and myself ('89), the amnion has had no such remote phylogenetic history, but has arisen more recently in response to certain purely mechanical conditions of development.

Nusbaum advances the opinion that the cellular envelopes of the insect embryo are homologous with the "dorsal organ" of Crustacea. The saddle-shaped "dorsal organ" of *Ligia* and *Oniscus* is regarded as the key to this homology, the two flaps which clasp the sides of the Isopod embryo being equivalent to undeveloped amnioserosal folds. But I have shown in the present paper that the indusium of *Xiphidium* is very probably the homologue of the crustacean "dorsal organ," and as there is besides a well developed amnion and serosa in *Xiphidium*, Nusbaum's hypothesis must fall to the ground. His assertion was certainly premature that the "deux séries des organes aussi charactéristiques que le sont l'organe dorsal et les enveloppes embryonnaires, s'excluent réciproquement dans les deux groupes des Arthropodes, c'est-à-dire chez les Trachéates et les Crustacés."

So far as the insect envelopes are concerned v. Kennel's views do not differ essentially from Nusbaum's. He likewise homologizes the crustacean "dorsal organ" and the Poduran "micropyle" with the Hexapod amnion and serosa. But he goes further and includes under the same homology the amnion of *Peripatus*, Scorpions and *Chelifer* and the chitinous envelopes of Myriopods. He supposes all these structures to represent remnants of the annelid trochophore. I feel confident that he has jumbled together at least three categories of organs which cannot be regarded as homologous *inter se*, viz.: (1) the series of structures typically represented by the Crustacean "dorsal organ"; (2) the cellular envelopes of insects;

(3) the chitinous cuticles. As stated above, the amnions of *Peripatus* and Scorpions probably also represent structures of independent origin and no wise homologous with the envelopes of insects. It is perhaps unnecessary to add that the reduction of all these structures to the annelid trochophore is in the present state of our knowledge little more than a wild guess.

Graber ('90) has criticized the view advanced by Will and myself, that the insect amnion arose by an invagination of the germ-band like that of some Myriopods (*Geophilus*). His contention is certainly in great measure well-founded. Still I believe that it does not affect the essential point of the hypothesis which implies that the amnioserosal fold is the mechanical result of a local induplication of the blastoderm due to rapid proliferation in a single layer of cells.

Ryder ('86) has sought a mechanical explanation for the amnion, and although his paper treats mainly of the vertebrate amnion, he evidently implies that the homonymous envelope of the Insecta had a similar origin. According to him "the amnion in all forms has arisen in consequence of the forces of growth resident in the embryo, encountering peripheral and external resistance either in the form of a rigid outer egg-shell (zona radiata) or decidua reflexa, or even the walls of the uterine cavity itself, supposing of course that a large vesicular blastoderm containing yolk has been formed by epiboly."

This view applies with little alteration to the Insecta. There is the vesicular one-layered blastoderm filled with yolk and the germ-band arising by rapid proliferation at one point. The resistance of the yolk being less than the external resistance of the tightly fitting chorion and vitelline membrane on the one hand combined with the peripheral resistance of the extra-embryonal blastoderm on the other, the germ-band is forced to invaginate. This invaginative process is favored by the displacement of yolk during its liquefaction and absorption by the growing embryo. We may suppose that this invagination which results in the formation of the amnioserosal fold, assumed a definite and specific character in different groups of insects.

Conditions similar to those to which the insect germ-band is subjected during its younger stages are often present in the ova and young of other animals, and would be expected to lead to the formation of structures similar to the insect amnion. And this is found to be the case. A hasty glance through the animal kingdom at once suggests a number of parallel instances: the invagination from which the Cestode head develops in the *Cysticercus;* the similar invagination in the larval Gordiid; the origin of the Nemertine in the *Pilidium;* the formation of the definitive trunk in *Aulastoma,* according to Bergh ('85); the development of the trunk and Scheitelplatte in *Sipunculus,* according to Hatschek ('84); the formation of the young Spatangid in the Pluteus, according to Metschnikoff, and the somewhat similar conditions in the development of the *Antedon,* according to Barrois ('88); the formation of the trunk in the *Actinotrocha* of *Phoronis* (E. B. Wilson '81); the development of the Polyzoan within the statoblast (Oka '91; Davenport '91). I need hardly say that the development of the amnion and serosa in vertebrates is a strictly analogous case. A case still more to the point, because occurring in the Insecta, is the formation of the imaginal disks. In this process we have all gradations till we reach the extreme in *Musca,* where the hollow disks whose inner walls bud forth the imaginal appendages are almost completely abstricted from the original hypodermis. The resistance of the chitinous cuticle of the larva in causing the invagination of the disks admits of easy observation. It certainly cannot be claimed that in all the different forms here enumerated genetic relationship lies at the bottom of the mutual agreement in the methods of forming the trunk or certain organs. On the contrary, everything goes to show that these similar methods in widely separated groups have been independently acquired under the stress of similar developmental conditions.

Perhaps the most difficult point to explain in the view here advanced, is the complete abstriction of the amnion from the serosa in nearly all insects. It is more natural to suppose that the inner envelope would remain continuous with the outer, so that the embryo could the more readily be everted

during revolution. The only explanation I have to offer, will be given in connection with a discussion of the movements of the germ-band. In that connection the variations in the development and amputation of the envelopes in the different groups of insects may also be treated to greater advantage.

2 *The Yolk.*

To my knowledge, the quantity of yolk in the insect egg has not been made the subject of comparative study. It has long been vaguely stated (*vide* Brauer, '69 and '70) that the eggs of Ametabolous insects contain relatively more yolk than the eggs of the Metabola. In other groups of animals (Crustacea, Annelida, Mollusca, Vertebrata), it is often observed that absence of yolk is correlated with free larval development, while in eggs provided with an abundance of yolk the larval stages are either lacking or considerably modified. This same law obtains also in the Hexapoda, though it can hardly be formulated so concisely as in other groups of animals. And this is not surprising when we stop to consider that, as regards complexity of organization, the difference between the simplest insect larvæ, such as those of the Muscidæ and their highly specialized imagines, is far from being as great as the differences between the trochophore and the Annelid, or the Nauplius and the crustacean.

Beginning with the Orthoptera we find that the egg is provided with an abundance of yolk, — the germ-band when first formed in most cases covers only a very small portion of its surface, and when it reaches its maximum length before revolution is no longer than, and usually not so long as, the egg.[1] The period of embryonic development is greatly prolonged; most of the species are monogoneutic and oviposit in the fall, the larvæ not hatching till the following spring or summer. There is practically no metamorphosis.

In the most highly metabolic insects (Muscidæ) on the other hand, the quantity of yolk is comparatively limited. The germ-band before revolution is nearly double the length of the egg,

[1] To this rule *Gryllotalpa* seems to be a noteworthy exception.

so that the head and tail ends nearly meet. Embryonic development is completed in a day, and the larva must pass through a complex metamorphosis to reach the imaginal state.

The chasm between these two extremes is bridged by the less metabolic insects (Coleoptera, Neuropotera, Lepidoptera, Hymenoptera, etc.). The quantity of yolk is intermediate between that of the Orthopteran and Dipteran egg. The germband, like that of the Muscidæ, is longer than the egg when it reaches its full length. But it is at this time much narrower than the yolk-mass, whereas in the Muscidæ it embraces nearly half the circumference of the yolk. The larvæ usually hatch after a period of ten to thirty days in a relatively more advanced stage of organization than Dipteran larvæ.

It is probable that the quality of the yolk is also an important factor in development. The yolk of the Orthoptera and Rhynchota is dense and resembles that of the crustacean and Arachnid egg, while the yolk of the Metabola seems to have a much looser molecular structure. Hence, bulk alone is no criterion of the amount of yolk in an insect's egg.

The view here advocated, that the eggs of the Ametabola contain more yolk than those of the Metabola, admits of some exceptions. Thus the 17-year locust (*Cicada septendecim*) is a large insect with incomplete metamorphosis, but it nevertheless produces a great number of very small eggs. This is, however, seen to be a greater advantage to the insect than the production of a few large eggs, when we consider the extremely long period of larval life and the vicissitudes to which the larvæ may be subjected during all this time. Similarly, *Meloë angusticollis* produces a great number of very small eggs, while the eggs of the smaller beetles (*Doryphora, e.g.*) are much larger. But *Meloë* is a parasite form, and probably only a few of its many offspring ever succeed in gaining access to the eggs of the bee. The larvæ, as shown by their hypermetamorphosis, are subjected to very varied conditions, and this would still further tend to reduce the number of successful individuals. As in anemophilous plants many germs are produced, but very few are destined ever to prosper. Many other exceptions to the general rule, like these two, are probably due to habits

which necessitate the production of a great number of ova at the expense of their size. The opposite exception occurs in the parasitic Pupipara, where the nourishment of the single larva within the parent is equivalent to the production of a large yolk-laden egg.[1]

The question naturally arises: Were the eggs of the primitive Insecta poor or rich in yolk? As all the evidence of comparative anatomy, embryology and paleontology goes to show that the Metabola are the more recent, the Ametabola the more ancient forms, we are justified in maintaining that primitive insects, or at any rate the primitive Pterygota supplied their eggs with a considerable quantity of yolk. At first sight the Apterygota, which have holoblastic eggs, would seem to constitute a serious obstacle to this view, but it must be remembered that total cleavage is not necessarily a criterion of paucity of yolk (witness Arachnida, Crustacea, and Myriopoda). Furthermore, the eggs of some Thysanura, *Anurida*, *e.g.* are provided with an abundance of yolk. Holoblastic cleavage in this group is probably a Myriopod trait, as was long ago suggested by Metschnikoff ('74). We might perhaps conclude that the superficial type of cleavage, like the embryonic en-

[1] The differences between the eggs of different insects with respect to the amount of yolk is systematically disregarded by Graber ('90). This is shown by his classification of germ-bands as microblastic and macroblastic, brachyblastic and tanyblastic. These distinctions are readily shown to be distinctions in the amount of yolk and not in the germ-band. Thus the just-established germ-bands of the Saltatory Orthoptera appear to be very small because the eggs contain an enormous quantity of yolk; while the germ-band of the Muscidæ appears correspondingly large on account of the small quantity of yolk. The amount of yolk fluctuates even within the limits of the single orders so that the newly-formed germ-bands appear to differ in length more than they really do. In the Orthoptera we have the following series in which the amount of yolk decreases, the germ-band in consequence appearing to increase: *Melanoplus*, *Mantis*, *Œcanthus*, *Gryllus*, *Xiphidium*, *Blatta*, (*?*) *Gryllotalpa*.

Graber's further classification of germ-bands as orthoblastic and ankyloblastic, or straight and curved, is equally artificial. In the great majority of cases the shape of the germ-band depends upon the yolk surface on which it arises, or over which it happens to grow. The uselessness of such a classification is also shown in the case of *Xiphidium* and *Orchelimum*, where the just-established germ-band is straight, but becomes curved in passing to the dorsal surface, and thereupon again becomes straight. To which of Graber's classes does this germ-band belong?

velopes and the wings, originated in the ancestral Pterygota. But Lemoine ('87) claims that the segmentation of the Poduran *Anurophorus laricis* approaches the superficial type, so that this latter may have had a still more remote origin. It is, however, hopeless to speculate on this subject till the eggs of many more Thysanura and Myriopoda, including the Symphyla, have been studied.

The relations of yolk-quantity to the movements of the embryo will be considered in the following paragraphs.

3. *Blastokinesis.*

According to Hallez ('85 and '86) "La cellule-oeuf possède la même orientation que l'organisme maternel qui l'a produite: elle a un pôle cephalique et un pôle caudal, un côté droit et un côte gauche, une face dorsale et une face ventrale; et ces différentes face de la cellule-oeuf coincident aux faces correspondantes de l'embryon." This law was founded on a study of the eggs of *Periplaneta*, *Hydrophilus* and *Locusta*, but it finds full support in the descriptions and figures of all investigators of insect development.[1] My own observations, based on some thirty different insects, accord perfectly with those of Hallez.

In most eggs the cephalic and caudal poles are readily distinguishable, the micropyle being usually located at or near the former. In exceptional cases, however, it is located at the caudal pole. There is frequently a slight flexure in the longitudinal axis of the egg, foreshadowing the dorsal and ventral, and consequently also the lateral regions of the mature embryo. The more nearly the egg approaches the spherical form, as in certain Lepidoptera and Coleoptera and in the Trichoptera, the more obscure become the relations of the egg-surfaces to the body-surfaces of the mature embryo. There is, however, every reason to suppose that these relations still exist.

The practical value of Hallez' law was shown in studying the *Xiphidium* egg; all the movements of the germ-band could

[1] The only exception is Ayers, who was undoubtedly mistaken in regard to the orientation of the young *Œcanthus* embryo.

at once be referred to the axis of the mature embryo. When the eggs of other insects are oriented in the same manner, it is seen that the germ-band invariably arises on the ventral surface of the yolk with its procephaleum directed towards the cephalic, and its tail towards the caudal pole. No matter what positions it may subsequently assume, it always returns to its original position before hatching. Frequently the germ-band, when newly formed, lies nearer the lower than the upper pole (*Calopteryx*, *Œcanthus*, *Stagmomantis*, *Hydrophilus*, etc.). The usual movements are very simple ; from a position of rest on the ventral surface of the egg, the germ-band moves through an arc till its body is completely inverted. Then it rests and again passes back through the same arc to its original position on the ventral yolk. These movements may be compared to the single vibration of a pendulum. The ascending movement I shall designate as *anatrepsis*, the descending as *katatrepsis*, the intervening resting stage as the *diapause*. The general term *blastokinesis* may be used to include all the oscillatory movements of the germ-band.

Inasmuch as the germ-bands in other Arthropods (Crustacea, Myriopoda, Arachnida, and Thysanura) exhibit no movements comparable to those of the lower Pterygota, and since, moreover, the insect germ-band is formed in exactly the same manner as that of other Arthropods and ultimately returns to its original position, no matter what oscillations may intervene, it is safe to infer that blastokinesis has been acquired within the Hexapod and probably even within the Pterygote group. We may also infer from the intimate relations of envelope-formation to blastokinesis in most forms, that both of these processes arose at about the same time.

No attempt has been made to account for the origin of blastokinesis. It has occurred to me that it may be due to causes of a purely physiological nature. The eggs of the primitive Pterygota were, as I have attempted to show, provided with a considerable amount of food yolk. Like their modern descendants they were probably also invested with dense chitinous envelopes. These must render the respiration of the embryo difficult as compared with embryonic respiration in annelids, mollusks and

vertebrates, or even as compared with the Crustacea, which usually have much thinner envelopes than insect eggs. Special provision is also made in many of the Crustacea for aerating the eggs. Now the cells of the rapidly growing insect embryo not only absorb and metabolize the yolk but also give off a certain amount of waste matter. That this is not wholly of a gaseous nature is seen in older embryos which have considerable accumulations of uric salts in the blood corpuscles and fat-body. Waste products are undoubtedly given off during the stages preceding anatrepsis, and probably permeate the yolk in the immediate neighborhood of the germ-band. As the oxidation of these waste products is very probably retarded by sluggish transpiration, and as growth under such conditions would be seriously impeded, we may suppose that the embryo has acquired the habit of moving to another part of the egg where the yolk is as yet unpolluted. Here it grows apace till the surrounding yolk is again charged with excreta. Growth is then temporarily suspended and the embryo moves back to the ventral surface. The embryo reaches a considerable size before katatrepsis, so that its rotation must cause a considerable circulation in the yolk bodies. This would also serve to aerate the yolk and to bring fresh pabulum in contact with the assimilating cells of the embryo. It may also be noted that in many insects the movements set in at critical periods of growth. Thus in *Xiphidium* anatrepsis occurs during the addition of new segments, and in many other forms it immediately precedes the formation of new segments. In the Orthoptera, katatrepsis usually occurs in the spring and is the signal for a decisive advance in the development of the heart, sexual organs, compound eyes, etc. During this period, also, the abortion of such rudimental structures as the pleuropodia, abdominal appendages and envelopes seems to be hastened. In short, the whole process of katatrepsis, at least in *Xiphidium*, has the aspect of rejuvenescence. It will be remembered that the amnion is formed just before or during anatrepsis. It is probable that the complete abstriction of this envelope from the serosa is a device for favoring the movements of the embryo. The germ-band is thereby set adrift on the yolk and enabled to

migrate to some other surface. This, of course, necessitates a secondary union of the envelopes previous to katatrepsis.

The hypothesis set forth in the preceding paragraphs is also supported indirectly by the fact that in the eggs of the Metabola which are less abundantly provided with yolk than the eggs of the Ametabola, blastokinesis is either faint or wanting. Aeration would be much less necessary in such small eggs. The lengthening and shortening movements seen in the embryos of the Metabola as well as in those of the Ametabola may suffice to keep the yolk circulating. The Lepidopteran germ-band, it is true, exhibits movements, but the eggs of these insects are laid in exposed situations and provided with unusually thick envelopes, so that the movements of the embryo, though differing widely from the typical blastokinesis of lower forms, have perhaps been independently acquired for a similar purpose.

I had intended to give a comparative description of blastokinesis in the different orders of insects but as the known facts have been recently summarized in a masterly manner by Korschelt and Heider ('92) I shall confine my remarks mainly to the Orthoptera. Although Graber, Ayers and others have studied representatives of this very important group, they have given but fragmentary and often inaccurate accounts of the relations of the embryo to the yolk-mass at different periods of development.

I may begin my account with the Saltatoria which comprise the three families Gryllidæ, Locustidæ and Acrididæ. As representatives of the first, *Gryllus luctuosus* and *Œcanthus niveus* were studied. In both of these insects as was pointed out at p. 42 the germ-band arises on the ventral surface of the yolk near the caudal pole. During the formation of the envelopes anatrepsis sets in and carries the germ-band to the dorsal surface where it rests through the winter in an inverted position with its head directed to the caudal and its tail to the cephalic pole. In the spring the envelopes over the head end first fuse and then rupture ; the embryo is thereupon everted and during katatrepsis passes around the caudal pole to regain its upright position on the ventral yolk. The envelopes during

this process are stripped back over, and finally drawn into the yolk, where they undergo dissolution when the body walls have met in the median dorsal line. The defects in Ayers' description of *Œcanthus* ('84) were pointed out at p. 43.

Gryllotalpa, the only other Gryllid, which has been studied, seems to differ considerably from *Gryllus* and *Œcanthus*. Examination of Korotneff's figures ('85) shows that this difference is probably more apparent than real. In his surface views, there is a wide gap between his Fig. 2, representing the egg in a preblastodermic stage, and his Fig. 3, representing quite an advanced embryo. One is thus left without any guide to the exact relation of the just-established germ-band to the yolk-surfaces. Korotneff's defective account of the formation of the germ-layers would seem to show that he did not study these early stages closely. It is obvious that *Gryllotalpa* is blastokinetic both from Korotneff's statement that the embryo moves during revolution and from his figures 5, 7, and 8, but the exact nature of the process is not clear. The possibility of the embryo's passing to the opposite surface of the egg is not precluded by the conditions seen in Figs. 7 and 8. Judging from *Gryllus* and *Œcanthus* I am inclined to think that the embryo exhibits both ana- and katatrepsis, but that Korotneff has overlooked the former and misinterpreted the latter movement.

In the Locustidæ, as represented by *Xiphidium* and *Orchelimum*, we find a modification of the blastokinetic process observed in *Gryllus*. Instead, however, of arising near the caudal pole, the germ-band is formed on the middle of the ventral surface, and instead of passing around the caudal pole during anatrepsis it passes through the yolk as if to reach the dorsal surface by a shorter path. Katatrepsis is essentially the same as in the Gryllidæ, the embryo passing around the caudal pole. This lack of coincidence in the anatreptic and katatreptic paths is one of the most striking peculiarities of Locustid development; since it is known to occur in no other insect. It is probable that the anatreptic embryo originally passed around the lower pole, but that owing to the formation of the embryo higher up on the ventral surface, and perhaps also to an acqui-

sition of yolk at the lower pole, this movement has been deflected.

Melanoplus femur-rubrum was studied as a representative of the Acrididæ. The germ-band is formed very near the caudal pole of the egg, but still on the convex ventral surface. During the formation of the envelopes the posterior end of the body grows around the pole onto the dorsal surface, while its head remains fixed at the pole. It is not until the germ-band has reached a stage corresponding to Stage F. in *Xiphidium* that its head leaves the pole and the whole body moves upward on the dorsal surface. It soon comes to a standstill and passes the winter in this inverted position. In the spring it moves back around the lower pole and, like the Gryllid and Locustid embryo in a corresponding stage, proceeds to lengthen and envelop the yolk till its head reaches the cephalic pole.

Packard ('83) seems to have been the first to study the development of Acridians (*Melanoplus spretus* and *M. atlanis*). But he had no conception of the true relations of the embryo to the yolk, as is shown by his Fig. 1, Pl. XVII, where the egg is depicted with the micropylar end uppermost. Leuckart ('55) long ago showed that the Acridian micropyle is located at the caudal pole. If the egg figured by Packard be inverted, it will represent the embryo on the point of undergoing katatrepsis.

The same error is committed by Graber in his accounts of *Stenobothrus variabilis* ('88, '90). Misled, like Packard, by the position of the micropyle, he has mistaken the caudal for the cephalic pole. To mean anything his figures must be inverted. As I have not yet studied the later stages of *Melanoplus* in section, I will not attempt to describe the details of katatrepsis. Graber claims to have observed that the pleural ectoderm, where it passes into the amnion proliferates a thin cell-lamella to form the dorsal wall, while the amnion remains intact and still covers the ventral face of the embryo. This account is not substantiated by his figures (1 and 2, Pl. I). The two thin cell-lamellæ extending over the dorsal surface have every appearance of being the walls of the heart, and therefore mesodermal, although it is difficult to see how this organ could be so completely formed in so early a stage. As

Graber has paid no attention to the movements of the *Stenobothrus* embryo, and as he most assuredly has not demonstrated from a careful study of the later stages that the lamella in question is really converted into the dorsal wall, I cannot attribute much value to his observation.

The foregoing observations go to show that the blastokinetic processes are essentially the same throughout the suborder Saltatoria. Each family presents certain deviations from the type, which is probably most closely adhered to in the Gryllidæ. Anatrepsis is aberrant in the Locustidæ, while the Acrididæ are aberrant in the tardy separation of the procephaleum from the lower pole. Notwithstanding these deviations the Saltatoria form a clearly circumscribed group embryologically as well as anatomically, and were it not for *Gryllotalpa* would be separated by a wide gap from all other Orthoptera. *Gryllotalpa* is a generalized form, as Brauer has pointed out from a study of its anatomical peculiarities ('86), and his conclusions are to some extent substantiated by the large size of the germband as compared with the yolk mass.

In the Cursoria, as represented by *Blatta germanica*, movements of the embryo are far less apparent. The germ-band never leaves the ventral surface, on the middle of which it first appears. I have shown, nevertheless ('89, text-figures, p. 348), that it moves down the yolk after the rupture of the envelopes till its tail reaches the lower pole. The tail then remains stationary, while the head gradually rises to the cephalic pole as the body walls develop and invest more and more of the yolk. Slight as are these movements, they nevertheless recall the blastokinesis of the Saltatoria. I would regard the movement of the whole *Blatta* embryo towards the caudal pole as anatreptic ; katatrepsis is probably represented only by the upward growth of the embryo. The very late occurrence of the former movement may be due to its rudimental character, since it is too weak to carry the germ-band around the caudal pole.

Few observations have been published on the relations of the embryo to the yolk in the Gressoria. In *Mantis*, as I have shown, the germ-band when first formed lies somewhat nearer the posterior than the anterior pole. The embryo never leaves

the ventral surface of the egg, but whether or not it exhibits any traces of blastokinesis my limited material will not enable me to decide, and Graber ('77), Bruce ('86), and Viallanes ('90[a], '90[b]), have contributed no observations bearing on this point. It is clear, nevertheless, that in its development, *Mantis* resembles *Blatta* more closely than either of these forms resemble the Saltatoria. This merely confirms the view which has long been held respecting the affinities of the Blattidæ and Mantidæ. From the structural similarity of the Phasmidæ and Mantidæ we may venture to infer a similarity of embryonic development.

It thus appears that the Orthoptera are clearly separable into two groups — the Saltatoria on the one hand and the Gressoria and Cursoria on the other. The Saltatoria are decidedly blastokinetic whereas the non-saltatory forms retain only faint reminiscences of blastokinesis (*Blatta*). I am inclined to believe that primitive embryological features have been preserved more faithfully in the Saltatoria than in other Orthoptera. That the habits of oviposition are more primitive in this group is shown by Brongniart's discovery of a fossil Blattid provided with an ovipositor ('89). Moreover, several features in the development of the Saltatoria show great conservatism, *e.g.* the retention of the indusium in the Locustidæ, the order in which the metameres arise, and the myriopod-like habitus of the *Xiphidium* embryo in Stage D.

Not only does a study of the Saltatoria throw light on the development of other Orthoptera, but it brings the order into closer union with the Odonata and Rhynchota. The blastokinesis of the Gryllidæ agrees closely with that of the Hydrocorisa among the Hemiptera — *e.g.* *Corixa*, as described by Metschnikoff ('66). *Ranatra* and *Zaitha* will bear even a closer comparison with the Gryllidæ. In the much elongated egg of the former, which has the cephalic pole marked by the pair of diverging pneumatic threads, the germ-band arises as usual on the ventral surface with its head directed upwards. As the envelopes develop it passes around the lower pole and finally assumes an inverted position on the dorsal surface. During katatrepsis it returns over the same path. The inclusion ob-

servable in *Corixa* and probably also in *Ranatra*, of a small quantity of yolk between the caudal amnion and the overlying serosa when the embryo first passes to the dorsal surface, is often observed in the Saltatoria. It is no great step to pass from the conditions seen in the Hydrocorisa to the "entoblastic" condition of other Hemiptera (*Pediculus*, *Aphis*, *Cicada*) and the Odonata (*Calopteryx*), where the germ-band instead of passing to the dorsal yolk during anatrepsis, comes to lie in the middle of the yolk, or even near the ventral surface (*Pyrrhocoris*). The Thysanoptera, as may be inferred from Jordan's brief statement ('88), the Corrodentia (Mallophaga) according to Melnikow ('69), and the Psocidæ, according to Packard ('84), are also referable to the "entoblastic" type. Concerning the embryonic development of the Plecoptera and Dermaptera nothing is known.

So far only the Homomorpha have been considered. The eggs of the Heteromorpha, as I have attempted to show, contain less yolk. Blastokinesis is nearly or quite lost in this more recent group, a fact that perhaps indirectly tells in favor of my view that the movements of the embryo have been acquired for the purpose of ventilating the yolk and supplying the growing embryo from time to time with fresh pabulum. The transition to the Heteromorpha is probably represented by the Ephemeridea. According to Burmeister's account of the development of *Palingenia horaria* (I quote from Zaddach, '54): "am dritten Tage, nachdem das Ei gelegt war, hatte sich der Keimstreif gebildet, der zungenförmig war, und sich über zwei drittel der Eilänge erstreckte, also in Form und Ausdehnung ganz dieselben Verhältnisse zeigte, wie im Phryganidenei." This may, perhaps, be taken to indicate that the Ephemeridea exhibit no blastokinesis; but the subject requires urgent investigation.

Among the Heteromorpha it is especially the Coleoptera which still show distinct though abortive movements of the germ-band. *Hydrophilus* may be taken as an example. As may be seen from Heider's figures, the germ-band forms on the lower ventral surface of the egg. As it grows in length, and the amnion is formed, the tail curls around the caudal pole on-

to the dorsal surface, but soon separates from the serosa so that a small amount of yolk is enclosed between the two envelopes. Later the yolk is expelled from this region and the envelopes become applied to each other. A true movement then sets in and carries the anterior portion of the germ-band forward up the ventral surface till the procephaleum overlaps the cephalic pole (*Cf.* Heider's figures, 4 *c*, 6 *a*, 7 *a* and 9, Pl. II. ('89). A certain similarity of these movements to those exhibited in *Blatta* leads me to believe that they represent a weakened blastokinesis.

Whether or not similar movements occur in the other so-called "ectoblastic" forms (Diptera, Hymenoptera, Siphonaptera, Neuroptera, Trichoptera) cannot be decided at present. If such movements occur at all they are probably exceedingly weak.

As stated above, the Lepidoptera have developed embryonic movements peculiar to themselves. In all the members of the order hitherto studied, the germ-band arises on the ventral surface of the egg, and its envelopes are formed while it is still in this position. As development proceeds the convex ventral surface of the germ-band, with its adherent amnion, moves back from the ventral serosa and soon comes to lie in the middle of the yolk. Hereupon the ventral surface of the embryo becomes concave, and its dorsal surface is applied to the dorsal serosa. I have already remarked that this movement of the embryo may have been independently developed for the same purely physiological purposes as blastokinesis in the Homomorpha. The fact that the movement is represented in the Trichoptera only by the change in flexure of the longitudinal embryonic axis, would seem to indicate that it has been acquired since the Lepidoptera diverged from the Trichopteroid ancestor.

Graber ('90) has recently made the interesting discovery that the Phytophagous Hymenoptera closely resemble the Lepidoptera in the movements of the embryo and in the amputation of the envelopes. This, taken together with the striking resemblance between the eruciform larvæ of the two groups, appears to point to a closer relationship than has usually been claimed.

While studying the movements of the embryo and the formation of the envelopes in the different orders and families of insects, with a view to testing the current classification, which is the outcome of a great amount of comparative anatomical and paleontological work, I have been especially impressed with two facts: First, the embryological data in no wise conflict with the generally accepted classification of Brauer. The developmental variations within limited groups are never greater than the post-embryonic differences in the members of the same groups. Usually there is great uniformity in embryological development between systematically allied insects of the same order; the wide gaps usually occur between the orders just where gaps have long been pointed out by comparative anatomy and paleontology. Second, developmental differences between members of different allied families of Orthoptera are greater than the differences between remotely related families in more recent orders. For example, the differences between a Locustid and an Acridian or a Locustid and a Gryllid embryo, or between any of the Saltatoria and the Blattidæ, or Mantidæ, are greater than the differences between an embryo Hydrophilid and a Chrysomelid, a Tabanid and a Chironomid, or a Bombycid and a Shingid. Frequently, it is true, the differences between the extremes in the higher orders are considerable, as between the Tenthredinidæ and the Proctotrupidæ among Hymenoptera, or the Chironomidæ and Muscidæ among Diptera. If any conclusions bearing on classification can be drawn from the few embryological data which I have collected, they refer to the ordinal value of the various Orthopteran families. It would appear that these groups have really more than family value. They are older than the families of more recent groups, and therefore exhibit greater divergence. The Rhynchota will probably be found to present conditions similar to the Orthoptera. There are certainly more considerable differences between the embryos of such forms as *Pyrrhocoris* and *Ranatra* than there are between the embryos of widely separated families among the Coleoptera.

4. *The Elimination of the Embryonic Envelopes.*

Anatrepsis and katatrepsis in the lower insect orders, or the completion of the envelopes and their rupture in the higher orders, are separated by a distinct interval, during which the germ-band undergoes a considerable development. But during this interval, the diapause, no change is noticeable in the envelopes themselves beyond a thinning of the amnion with the increased growth of the embryo. The elimination of the envelopes is preceded by katatrepsis just as their formation was preceded or accompanied by anatrepsis. This elimination is immediately followed by the completion of the dorsal body-wall and may take place in a variety of ways. Korschelt and Heider ('92) distinguish the following types in this process:

1. The amnion and serosa become continuous and, after the eversion of the embryo, are drawn back over the yolk to form a single layer of cells. As the dorsad growth of the body-walls proceeds, both envelopes are drawn together and pushed into the yolk to form a sack or longitudinal tube which is ultimately enclosed by the walls of the mesenteron and absorbed. To this type belong the Odonata, Rhynchota, some Orthoptera (*Blatta*, *Œcanthus*, *Gryllotalpa*) and some Coleoptera (*Hydrophilus*).

2. The serosa is shed from the yolk and the amnion alone contracts on the dorsal surface preparatory to being drawn into the yolk and absorbed. (Certain Coleoptera, *e.g. Doryphora*.)

3. The serosa alone is agglomerated and drawn into the dorsal yolk, the amnion being cast off. (Certain Diptera [*Chironomus*] and Trichoptera.)

4. Both envelopes are shed. (Lepidoptera and certain Hymenoptera.)

In *Xiphidium* we may perhaps recognize a fifth type, in which as in the fourth, both amnion and serosa are shed. But while the serosa is in great part shed as a simple membrane, the indusium which is a modified portion of the serosa, together with the amnion is drawn together in a mass and cut off from the embryo. It is more than probable that other types of envelope elimination will be discovered when more forms have

been studied. *Musca* may perhaps be regarded as representing a distinct type, since in this highly modified form the rudimental amnion and the serosa are neither shed nor agglomerated and engulfed in the yolk, but are supposed to form the definitive body-wall. (Kowalewsky, '86; Graber, '89.)

It is clear that the revolution of the insect embryo includes three distinct processes: first, the eversion and katatrepsis of the germ-band; second, the formation of the dorsal walls; and third, the elimination of the envelopes. The mechanical cause of eversion and katatrepsis is probably a contraction on the part of the envelopes after their fusion and rupture over the ventral surface of the embryo. After the embryo is everted from the amniotic cavity, or exposed after the rupture of the amnion and serosa, these envelopes temporarily form the dorsal covering of the yolk. Do they ever form the definitive dorsal body-wall? For both envelopes this is claimed to be the case only in *Musca*. In all other insects the serosa, at least, takes no part in forming the permanent body-wall, as it is either shed or engulfed in the yolk. The question is, therefore, restricted to the fate of the amnion. In many insects (Lepidoptera, Hymenoptera Phytophaga, some Diptera and Coleoptera), it has been shown that the amnion takes no part in the formation of the definitive body-wall, although a decision on this point is rendered difficult by the fact that no hard and fast line can be drawn between the ectoderm of the germ-band and the cells of the amnion. In other insects the decision is even more difficult. Still, I may say that I have seen nothing in the insects I have studied, to convince me that the amnion is converted into a portion of the permanent body-wall. Even in *Musca* it seems probable that the amnion and serosa only temporarily function as the body-wall, and that their cells are ultimately replaced by true ectodermal elements from the germ-band. In *Blatta* and *Xiphidium* I have seen appearances which lead me to believe that at least a part of the amnion may be eliminated by such a process of cell-substitution. I incline, therefore, to the views of Korschelt and Heider ('92), who hold that the envelopes are probably completely eliminated, and that the entire body-wall is derived from the ectoderm of the germ-band.

If this be the correct view, it follows that the dorsal body-wall is formed in essentially the same manner in all insects — by a growth and meeting of the germ-band edges. This process is, therefore, remarkably simple and uniform compared with the processes whereby the envelopes are eliminated. The great variability in the latter case has been dwelt on by Graber ('88) in a paper devoted to dorsal-wall formation in the Insecta. After reviewing all the literature on the subject and contributing many new facts, he proceeds to base a classification of the insects hitherto studied, on the "Keimhüllenzustände." He finds some fault with the current classification on the ground that insects which systematists regard as closely related often present great differences in their respective methods of dorsal-wall formation, whereas remotely related insects often agree very closely in this respect. Thus *Lina* and *Hydrophilus* differ more than *Hydrophilus* and *Œcanthus* in the processes whereby the dorsal-wall is formed. In considering Graber's views, I may pass over the awkward and kakophonous nomenclature which he has introduced, to what I regard as his main error, *viz.* the superficial analysis of his subject. Graber's term "Keimhüllenzustände," I take it, includes the formation of the envelopes as well as their condition preceding and during their elimination. Now I have attempted to show that there is nothing in the formation of the envelopes nor in the concomitant anatrepsis of the germ-band in the different insect orders to conflict with the current classification. Nor is there anything in the closure of the dorsal-wall in different groups — restricting this term to the confluence of the pleural edges of the germ-band — to support Graber's conclusion. His statement must therefore be restricted to the elimination processes. That these are highly variable must be admitted, but they are probably of very little taxonomic value, as Graber would probably have observed, had he attempted to account for the wide differences in allied forms and the agreement of remotely related species. It is my opinion that this high degree of variability in the elimination process is to be traced to the same causes as the variability of the indusium, viz., the rudimental character of the envelopes. Up to the close of the diapause

the envelopes subserve a distinct function, but as soon as the germ-band has invested the yolk with its own ectoderm, they have become functionless, or rudimental. Long before this time, in fact ever since their completion, the envelopes show no traces of cell-division. Moreover, their involution into the yolk or complete shedding shows conclusively that their morphological value is at this time reduced to *nil*. Whether both envelopes are shed instead of being drawn into the yolk, or whether one is shed and the other drawn into the yolk, may depend to some extent on the ease with which the pleural folds can close without their temporary assistance. But which of these processes shall occur in a given insect is probably a matter of no vital importance to the embryo, and has probably played no rôle in the struggle for existence. The involution of the envelopes, it is true, may add assimilable matter to the embryo, but enough energy to counterbalance this addition is probably consumed in metabolizing the dead cells. Hence the adoption of this process may be of no greater advantage to the embryo than the complete sloughing of the useless envelopes.

The insect envelopes, therefore, present only another case of an organ which has become specialized for a particular function at the expense of its formative power. This same phenomenon recurs in insect ontogeny. During cleavage certain cells are segregated for the express function of yolk-metabolization (vitellophags), while the remaining cells go to form the blastoderm. Later the cells of the blastoderm separate into those of the germ-band proper and those of the specialized envelopes. Still later, if the insect be metabolic, another splitting occurs, a portion of the hypodermis being set aside in the form of the imaginal disks to supplant the specialized primitive larval hypodermis. The formative material of the insect, like that of other organisms, thus undergoes a successive splitting into a specialized and a comparatively non-specialized portion. The former, being incapable of metamorphosis, is cast off or broken down, while the latter persists until a new segregation takes place. The analogy of this process to that occurring in rhizomatous plants, Polyzoa, etc., need not be pointed out in detail.

V. Neurogenesis in the Insecta.

1. *The Nerve-cord.*

The first traces of the central nervous system of *Xiphidium* make their appearance at a very early stage, before the blastopore is closed and while the envelopes are still incomplete. In this stage (Fig. 2) surface preparations made according to the methods given in the latter part of this paper, show a number of pale spots scattered over the procephalic lobes. They frequently occur also in the maxillary region, and, were it possible to remove the amnio-serosal fold without injuring the surface of the germ-band, would probably be found to extend still further caudad. The meaning of these spots is apparent when sections of embryos in Stages B–D are examined. In a transverse section (Fig. 25) through the middle of the abdomen of an embryo in Stage D, the ectoderm, which bulges out somewhat on either side of the median line, is seen to consist of two kinds of elements. First, there are a few large, clear, polygonal cells with spherical nuclei (*nb.*), lying in the deeper portion of the layer; and second, a much greater number of small and more deeply stainable cells (*db.*), differing in no essential respect from the cells forming the remainder of the ectodermal layer, such as the appendages. The latter cells have smaller, oval or cuneate nuclei, which appear to contain more chromatin than the large inner cells. While the small cells form a continuous layer, the large elements make their appearance singly or in small clusters, as seen in the figure. It is these pale clusters underlying the darker cells which produce the pale spots seen from the surface.

The large pale cells may be called neuroblasts — since it is they that give rise to the purely nervous elements of the cord.[1]

[1] The term "neuroblast" was originally used by Whitman ('**78** and '**87**) to designate the two offspring of the large posterior macromere of the *Clepsine* egg, which give rise by a process of budding to two rows of cells — the "neural rows." From these rows the nerve cord arises. His ('**89**) subsequently employed the same term "neuroblast" to designate such of the offspring of the "Keimzellen" as give rise directly by differentiation and not by further divisions to the ganglion-cells, or, to use Waldeyer's term, to the neurons of the vertebrate central nervous system. More properly the term would have been applied to the "Keimzellen" themselves, and by mistake it has been thus used by at least one recent writer (C. L. Herrick, '**92**, p. 430, Fig. 10). Haeckel (Anthropogenie, 4th ed. p. 268, '**91**) uses 'neuroblast' in the sense of ectoderm in general.

The remaining cells which cover the neuroblasts and extend down between them in the median line, give rise to purely integumental structures and may therefore be called dermatoblasts. The two thickenings of the ectoderm are to become the lateral cords (Seitenstränge). They extend from the anterior edge of the eleventh abdominal segment, just in front of the anus, to the mouth, where they diverge and pass without interruption into the brain. The groove which separates the lateral cords and which is very faint in Fig. 25, is the neural furrow (Primitivrinne). It appears soon after the closure of the blastopore and takes the place of this depression. It is deepest anteriorly.

All the neural structures develop in an anteroposterior direction, beginning with the brain; hence different stages in the development of the lateral cords may be studied in the same embryo. Fig. 26 shows a section passing through the first abdominal segment of the embryo from which the section in Fig. 25 was taken. Here we see a distinct advance in structure. The neural furrow (*n.g.*) is more clearly marked and the neuroblasts (*nb.*), four in either lateral cord, have arranged themselves side by side in a regular layer in the deepest portion of the ectoderm. Over them the dermatoblasts (*db.*) also form a single regular layer, while the cells lying in the median line on either side of the neural furrow have grown more elongate. Sections further forward show essentially the same conditions — the neuroblasts which were at first differentiated as small clusters or as isolated cells, have arranged themselves throughout the anterior portion of the embryo as an even layer entad to the dermatoblasts.

The further changes in the development of the nerve-cord, are brought about — first, by a proliferation of the neuroblasts ; second, by a proliferation of the dermatoblasts and a deepening of the neural furrow; third, by the development of the median cord; fourth, by the formation of the connectives and commissures, and fifth, by the development of the neurilemmata. These changes which occur simultaneously may be described singly for the sake of convenience.

As cross-sections show, the neuroblasts are arranged in from

3–5 longitudinal rows in either lateral cord. In surface view these rows may often be followed through one or two segments as continuous strings of cells. I assume that there were originally four of these rows, but that owing to the pressure exerted by the developing appendages on the lateral edges of the cords and to a more rapid growth of the neuroblasts than of the germ-band, the primitive regular arrangement has been considerably obscured. The neuroblasts are polygonal in outline from mutual pressure. When they divide, as they very soon do, their spindle axes are directed at right-angles to the surface of the body. As soon as one cell has been given off, the nucleus rests for a short time and then again divides in the same direction. This process continuing, a column of cells is budded off from each neuroblast and stands at right angles to the surface of the germ-band. The divisions do not take place simultaneously in all the cells although corresponding neuroblasts in either cord will frequently be found in the same phase of caryokinesis, especially in the earlier stages of their proliferation. A section (Fig. 27) through the first maxillary segment of an embryo in Stage F shows that each of the eight neuroblasts has produced a row of daughter-cells. The large succulent mother-cells are evenly rounded on their outer surfaces which are overlaid by the dermatoblasts. Their inner faces are flat or concave and in every case closely applied to the latest daughter-cell. The nuclei of the mother-cells are spheroidal and take no deeper stain than the pale succulent cytoplasm which surrounds them. The neuroblasts are in all essential respects typical proliferating cells like the terminal cells in plant-shoots and the teloblasts of annelids. The daughter-cells (g^1) are at first characterized by their small size, cuneate outline and deep stain. Their nuclei are considerably flattened, probably from mutual pressure. These characters are retained by the daughter-cells till they have been pushed some distance from the neuroblast by later offspring, when they become larger and considerably paler and assume the appearance of definitive ganglion cells (g^2).

Turning now to a somewhat older embryo (Stage G, Fig. 28) we see that the columns of daughter-cells have greatly increased

in length, while the neuroblasts remain to all appearances unaltered. The increase in the number of daughter-cells is so great that they are forced to arrange themselves in several rows. In the figure this is best shown in the progeny of the innermost neuroblasts, and the tapering columns there formed may be regarded as typical.

In my preliminary note ('91c) I held that the daughter-cells themselves divide to form the multiple rows in each pillar. I incline to think that I was mistaken on this point. The daughter-cells probably never divide but are directly converted into ganglion cells. All reproductive powers seem to be confined to the neuroblasts. Some of the nuclei of the daughter-cells exhibit peculiar chromatic structures which I may have mistaken for caryokinetic figures; this being an easy error to make in the case of small cells killed by means of heat, since the achromatic portions of the spindles are obliterated by this method.

The last stages in the proliferation of the neuroblasts are shown in Fig. 31, which is taken from an advanced embryo (Stage J). The columnar arrangement is no longer visible since the individual cells are now converted into the definitive ganglionic elements. On the outer periphery of the ganglia, however, neuroblasts are still to be found, and extending from them short series of small flattened cells (g''), their latest progeny, still distinguishable from the ganglion cells by their deeper stain. It will be noted that these cells, which like their precursors will become ganglion cells, are no longer budded off at right angles to the surface of the nerve-cord but parallel to it, a condition undoubtedly due to a lack of space. Finally the neuroblasts stop proliferating and shrink to the size of their progeny. Their chromatin then shows signs of senility. Beyond this point I have been unable to trace them satisfactorily. They are probably broken down and absorbed by the growing ganglion cells. Some of them may persist as ganglion cells of a particular character and function, though I deem this improbable.

The dermatoblasts play an important part in the development of the ventral nerve-cord, as will be seen by returning to the younger stages. We left these cells as a layer covering

the neuroblasts and continuous laterally with the general ectoderm. In the median line they extend to the deepest portion of that germ-layer in the form of a few compressed cells (Fig. 26, *db.*). These compressed cells form the walls and bottom of the neural furrow. The proliferation of the neuroblasts has caused the lateral cords to bulge out enormously (Fig. 27), so that the dermatoblastic layer becomes stretched and attenuated. Such divisions as occur in the cells of this layer seem to be confined to the outer surface and do not extend into the furrow. The spindle axes lie parallel to the surface, as shown at *nb.* The bulging of the lateral cords naturally brings about a deepening of the neural furrow, since the cells at its bottom have a fixed attachment. At this point in Fig. 27 there is seen a triangular cell-mass, capped by a single large element (*mnb.*), a true neuroblast which resembles in nearly all respects the neuroblasts of the lateral cords. Its more pyramidal outline is obviously the result of its position between the converging walls of the furrow. To the same mechanical cause is due the shape of the cell-mass, which consists of the heaped up daughter-cells of the neuroblast. Inasmuch as the proliferating cell occurs in the median line, and together with its offspring and the dermatoblastic cells of the median furrow, is equivalent to the "Mittelstrang" of authors, I shall call it the median-cord neuroblast. Its exact origin I have not been able to determine. To judge from the number of cells which it has given off it must have begun to proliferate at about the same time as the lateral-cord neuroblasts. There can be no doubt, it seems to me, that it originated as a polygonal ectoderm cell like the lateral cells seen in Figs. 25 and 26, but whether it was originally median in position or arose unilaterally I am unable to decide. The pale surface spots of embryos in Stage B show that neuroblasts are arising at a time when the blastopore occupies the position of the neural furrow and hence, if the median cells are median in position from the first, they must arise somewhat later than their sister neuroblasts.

There is one important difference in the arrangement of the mother-cells of the lateral and median cord. Whereas the former, as has been stated, form continuous though irregular rows

from mouth to anus, the latter constitute an interrupted series between the same two points. They are single, isolated cells, which occur only intersegmentally. That such is their distribution may be distinctly seen in frontal sections like the one represented in Fig. 30. This section passes through the first to fifth abdominal segments at the level of the median cord neuroblasts (*mnb.*), which are seen to lie distinctly between the segments, where the walls of the neural furrow dilate at intervals for their accommodation. At first the daughter-cells are given off in the same direction as those of the lateral cords, but soon the triangular space to which they are confined will no longer contain the older cells of the series and these are pushed along the floor of the neural furrow. This produces an angular flexure in the cell-column, but later the whole mass, including the neuroblast, assumes a horizontal position. This change in the position of the median cell-mass is seen to have taken place in the median sagittal section from an embryo in Stage G (Fig. 29). The neuroblast (*mnb*) is in each segment directed caudad, while the mass of small and deeply stainable daughter-cells (*mg*) is wedged in under the commissures. The section passes through the sub-œsophageal ganglion, which consists of the fused ganglia of the mandibular and both maxillary segments (*md. g; mx.* g^1; *mx.* g^2), and through the pro- and mesothoracic ganglia (*p.* g^1; *p.* g^2). Transverse furrows (*i.* g^1; *i.* g^2), which I shall consider later, separate the unfused ganglia from one another, and as the median cord cells lie in front of these furrows, they must be regarded as belonging not to the intersegmental region of the ectoderm, but to the posterior portions of the separate ganglia. Each ganglion possesses a median cord neuroblast, so that, beginning with the mandibular, which is the first ganglion in the nerve-cord proper, and ending with the tenth abdominal, there are in all sixteen median mother-cells. Each of these, after producing its quota of ganglionic elements, deteriorates in the same way as the mother-cells of the lateral cords.

The development of the Punktsubstanz may be readily followed in *Xiphidium*. It arises in each ganglion as two separate masses. Each of the daughter-cells of the lateral neuroblasts

sends out a cytoplasmic process which soon ramifies. The mass of fibres thus formed increases in size very probably by the addition of further ramifications till the Punktsubstanz is definitely established as a scarcely stainable body, lying on either side of the median line in the deepest portion of the lateral cord (Fig. 27, *p.s.*) In its earliest stages the formation of the substance is easily followed, but very soon the felted fibres become too dense for analysis by ordinary methods of investigation. It is only after a distinct mass is formed in either half of a ganglion that the longitudinal commissures, or connectives as they are best called, make their appearance, and unite the hitherto isolated centres in two longitudinal series. Very soon the transverse commissures, or commissures proper, of which *Xiphidium*, like all other insects, has two in each segment, make their appearance. The daughter-cells of the median cord neuroblasts take no part in the formation of the anterior commissure. Whether they contribute fibres to the posterior commissure or not, I must for the present leave undecided. I have seen no evidence in the median cord of a distinct and isolated Punktsubstanz centre, such as is described and figured by Graber for some Coleoptera ('90) Pl. V, Fig. 66). I deem it more probable that in *Xiphidium* the commissures arise wholly from the Punktsubstanz masses of the lateral cords. Both commissures are distinctly seen in cross section in Fig. 29.

The connectives and commissures incompletely divide the cellular portion of each ganglion into five parts, — two lying laterad to the connectives and a median series of three smaller portions separated from one another by the two commissures. The former may be called lateral gangliomeres, and the three median portions respectively the anterior, central, and posterior gangliomere.[1] Of the median divisions the posterior is distinctly the largest from the first. This is due to its being formed in great part by the progeny of the median neuroblast, whereas the anterior and central gangliomeres consist of a comparatively small number of cells, contributed by the lateral neuroblasts.

[1] These are equivalent to Graber's laterale Zellenlager, vorderes, centrales, and hinteres Medianlager.

It is not till after the commissures and connectives are formed that the inter-ganglionic regions become clearly marked out. Throughout the early stages, in fact till the embryo reaches the ventral surface of the egg (Stage J), the ganglia are as long as their respective segments and are separated from one another only by the intersegmental constrictions. These have grown very deep in Stage G, especially in the thoracic and abdominal regions. In the median line, as shown in sagittal section (Fig. 29, *i.g*^{1}, *i.g*^{2}.), they form deep tubular ingrowths which may be called furcal pits. Since these pits are median in position they are to be regarded as differentiated interganglionic portions of the neural furrow. They therefore belong to the median cord. They are not found between the mandibular and first maxillary, nor between this and the second maxillary ganglion, and are also wanting between the eighth and ninth, and ninth and tenth abdominal ganglia. Evidently their absence in these cases is due to early fusion to form the infraœsophageal and last abdominal ganglion. In the thoracic segments the furcal pits are converted into chitinous apodematous structures which give attachment to some of the leg-muscles. It is interesting to note that in the abdomen also furcal pits are distinctly developed as late as Stage K. Here, too, they serve for the attachment of a few weak muscle-like structures, which run from their tips to points in the adjacent abdominal wall, perhaps corresponding to the insertions of the rudimental appendages. Later both muscle-like cords and abdominal furcæ disappear, — the latter by a very simple process. It will be remembered that at this time the embryo is growing in length and continually covering more and more of the yolk. The tail end is practically fixed at the lower pole of the egg, while the head slowly moves upwards. The body-wall is thus stretched in both a longitudinal and lateral direction. Hence the intersegmental constrictions, so deep in Stage J, must gradually become shallower, and the furcal pits, which are nothing but portions of these constrictions, are drawn out from between the connectives to form part of the sternal integument. The stretching not only draws out the folds in the embryonic body-wall, but also reduces it to a much thinner layer

of cells. The length of the individual segments is thereby greatly increased and the nerve cord, which is firmly attached in the infraœsophageal region and more loosely in the terminal abdominal segments, is compelled to lengthen. The separate ganglia, besides assuming a somewhat fusiform outline, are scarcely affected by this traction, whereas the connectives are drawn out into thin threads denuded of all ganglionic cells and covered only by the neurilemma.

The presence in the abdomen of temporary furcal pits corresponding to the persistent furcæ of the thorax admits of an easy explanation, if we take these structures to be correlated with the development of ambulatory appendages. The temporary abdominal appendages have usually been regarded as the rudiments of once functional walking-legs, and they are still so well preserved in the Orthoptera that it need not surprise us to find traces of correlated structures which served for the attachment of some of their muscles.

The progeny of the median neuroblast together with the interganglionic portion of the neural furrow have been accounted for; the former becoming the posterior gangliomere, the latter a portion of the sternal integument ; but I have not yet accounted for the remaining portion of the median cord — *viz.* the intraganglionic walls of the neural furrow. This portion of the groove is crossed by the two commissures and separates those portions of the lateral cord which will ultimately constitute the anterior and central gangliomeres. Its cells are of an epithelial nature. Those of the opposite walls of the furrow become applied to one another by the swelling of the lateral cords. The lumen is thereby obliterated though its walls are still continuous on the outer surface of the ganglion with the integumental ectoderm. The two lips of the furrow finally fuse and the ganglion together with the portion of the furrow included between its two halves is liberated from the ectoderm. It is these epithelial walls thus set free from the integument which appear to give rise to the outer and inner neurilemmata. Both these neural envelopes are ectodermal ; there are no traces of mesodermal structures taking any part in their forma-

tion and it seems to me that they can have only two possible sources — they either arise from some of the progeny of the neuroblasts or from the intraganglionic portion of the median cord. I deem it highly improbable that they should arise from the former source, since the daughter-cells of the neuroblasts have every appearance of being early specialized as ganglion cells. Furthermore, the cells of the neurilemmata when they definitely appear, closely resemble the cells of the neural furrow both in size and in the great avidity with which they take the stain. The outer neurilemma covers first the inner surface of the ganglion — then the outer or neuroblastic surface; — the thin cellular membrane apparently progressing laterad in either case and meeting near the origin of the nerve-trunks. The inner neurilemma, which envelops the Punktsubstanz is completed before the outer envelope. Histologically both envelopes resemble each other in every respect.

The fusions of ganglia in the nerve-cord take place gradually and may be easily followed in *Xiphidium*. Several stages of these fusions are represented in Fig. VII, A–D. In Fig. A, the nerve-cord is shown much as it appears in Stage F. The ganglia form an unbroken series from mouth to anus. The connectives are very short and not as yet distinguishable from the surface. Fig. B, is taken from an embryo just turning the lower pole (Stage H). Here the mandibular and two maxillary ganglia, and also the three terminal abdominal ganglia still remain as in the preceding stage, while the other ganglia are being drawn apart by the stretching of the embryo, so as to show their short connectives. In Fig. C, the subœsophageal and last abdominal ganglia are established as two fused masses. The number of ganglia comprising each of these masses may still be easily determined by counting the commissures. It will be noticed that in this stage the first abdominal is closely approximated to the metathoracic ganglion and that the second and third abdominal also lie close together. Between the other ganglia the connectives have lengthened. In the later stage represented in Fig. D, the connectives are still longer; the first abdominal ganglion has fused with the metathoracic, and the second and third abdominal form a single mass.

These fusions become more intimate as the time for hatching approaches, so that the ventral cord finally consists of only ten ganglia instead of sixteen, the original number.

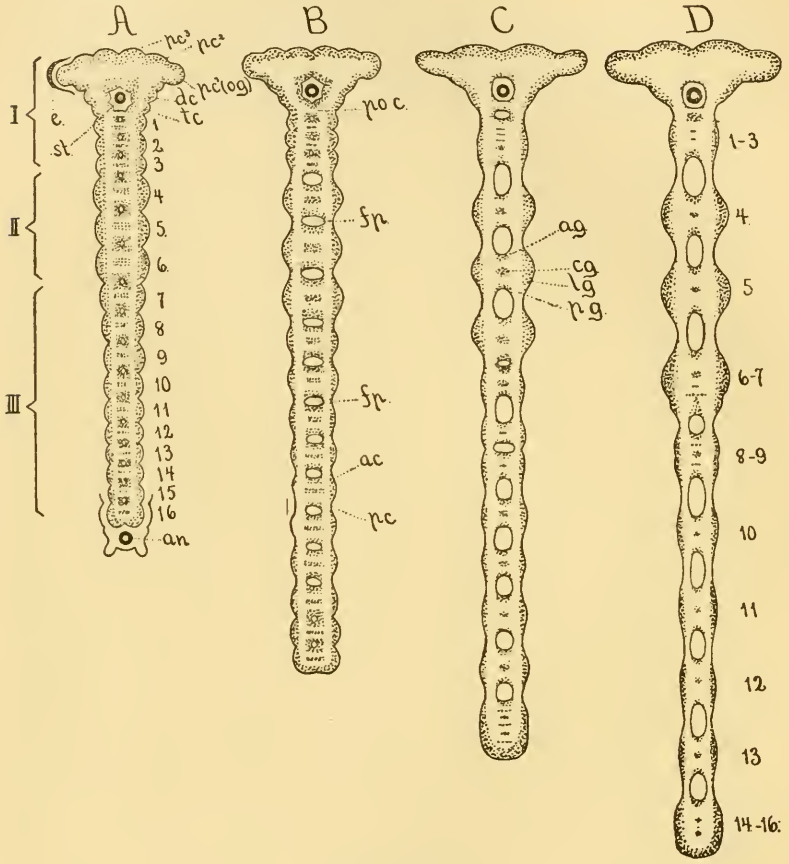

FIG. VII.

A–D. Diagrams of four consecutive stages in the development of the brain and nerve-chain of the *Xiphidium* embryo. I, cephalic; II, thoracic; III, abdominal region; *st.*, stomodæum; *an.*, anus; *e*, optic plate; pc^1(*o.g*), first protocerebral lobe, or optic ganglion; pc^2, pc^3, second and third protocerebral lobes; *dc*, deutocerebrum; *tc.*, tritocerebrum; 1–16, the sixteen postoral ganglia; *po.c.*, postoral commissure; *fp.*, furcal pit; *ac.*, anterior; *pc.*, posterior ganglionic commissure; *ag.*, anterior; *pg.*, posterior; *cg.*, central; *lg.*, lateral gangliomeres.

The description of the ventral nerve-cord of *Xiphidium* here given applies equally well to the other Orthoptera which I have studied (*Blatta germanica, Melanoplus femur-rubrum*). The

points in which the Blattid and Acridian nerve-cord differ from that of the Locustid are so insignificant that I need not burden the reader with their enumeration. I will stop to mention only two peculiarities in *Blatta*. Here I fail to detect the pale spots in the "slipper" stage of the germ-band, and sections show that the neuroblasts do not differentiate as early as they do in *Xiphidium*. They are, however, readily detected in late stages, when they stand out with even greater distinctness than in the Locustid. The median cord neuroblasts, though present and occupying positions corresponding to their homologues in *Xiphidium*, are more difficult to trace, probably on account of the smaller size of the embryo.

Neuroblasts, or cells of a similar character have been described and figured by a number of investigators of Arthropod development. Perhaps the earliest mention of these cells is to be found in Reichenbach's beautiful *Astacus* monograph ('86), where the nerve-cord is described as consisting in an early stage of two kinds of cells — a few large pale elements arranged in a single layer and confined to the periphery, and a much greater number of small and more deeply stainable cells forming the bulk of the ganglia. The developing ganglia of the cray-fish resemble the ganglia of the Orthoptera in many particulars. The number of large cells in the lateral cords in Reichenbach's figures (notably his figures 114-133) is 3-6, the average being 4 or 5, the same as in *Xiphidium*, *Blatta*, etc. Furthermore the ganglia of *Astacus* show a foliated arrangement of the smaller cells, which is not unlike the condition seen in the older ganglia of the Orthoptera. Some of the figures (188 and 189 for example) show a single neuroblast-like cell surmounting the median cord cell-mass. There are, however, two points in Reichenbach's work, which throw some doubt on the homology of his large cells with the neuroblasts of the Orthoptera. First, Reichenbach neither figures nor describes these cells as dividing to form ganglion cells. This negative observation, however, loses much of its force when we consider that caryokinetic figures are singularly absent from all of Reichenbach's figures, excepting his surface views of young embryos, and when we recall the fact that amitotic

division is a very general phenomenon in the Crustacea (according to Carnoy, '85). A more serious objection to the homology under consideration is Reichenbach's statement that the large cells 'gehen schliesslich in die von Leydig, Dietl, Krieger und anderen beschriebenen, grossen Ganglienzellen über.' This, too, is an objection only if the neuroblasts really degenerate, a point on which I am still doubtful.

Nusbaum found huge succulent cells in the young nerve cord of the embryo *Mysis* ('87). He compares them with the large cells of Reichenbach and believes that they have a similar fate. Similar cells were also observed in the brain of *Oniscus murarius* "pendant les stades relativement jeunes." On one point only does he add to Reichenbach's observations: he depicts (Fig. 78) a caryokinetic figure, which from its size and position must be referred to one of the large cells. Its spindle axis is directed at right angles to the surface of the body. This observation, small and incidental as it is, would tend to show that the large cells proliferate as in the Orthoptera. I am inclined to think that a renewed study of the Crustacean nerve cord will show that the ganglion cells are budded forth from the large cells and that these are equivalent to the insect neuroblasts.

Korotneff ('85) was the first to find gangliogenic cells in the Insecta. At p. 589 in his description of the Gryllotalpa embryo he says: "Einige der Ektodermzellen, welche die Nervenauftreibung bedecken, fangen an zu wachsen, ihre Kerne vergrössern sich bedeutend und zeigen dabei eine karyokinetische Figur. Grösstentheils sind diese Zellen (Ganglien) so angeordnet, dass einer einfachen platten Ektodermzelle eine wachsende Neuroektodermzelle folgt. Hat sie eine bestimmte Grösse erreicht, so sinkt jede wachsende Zelle in die Tiefe des Ektoderms und wird von den benachbarten, unveränderten Zellen bedeckt. Jede Ganglienzelle theilt sich dabei, eine ganze Folge von neuentstandenen Zellen bildend, nur an der Fig. 60 ist leicht zu unterscheiden, welche Gruppe von Zellen der oben gelegenen Ganglienzelle entspricht. Durch eine solche Vermehrung von Zellen wird der Nervenstrang mehr und mehr in die Höhe getrieben." The Fig. 60 referred

to in this description is taken from a stage corresponding to my Fig. 28. Both in this figure and in Fig. 61 he represents four neuroblasts in one of the lateral cords. Korotneff seems not to have seen the early stages of proliferation.

In the developing nerve-cord of *Doryphora* I observed ('89, p. 366) that "the outer layer of cells continuous with the hypodermis stands off somewhat from the ganglionic thickenings, leaving a space which is in early stages occupied by several large, clear, oval cells which divide rapidly by caryokinesis, and might be called *ganglioblasts*, as the products of their divisions reinforce the mass of ganglion cells." In my figures the polar-axes of the neuroblast spindles lie parallel to the surface of the ganglia. Re-examination of my preparations has convinced me that this observation is essentially correct. I find also that the newly-formed daughter-cells of the neuroblasts occasionally divide caryokinetically and thus give rise to further generations of daughter-cells. The daughter-cells are not budded forth in regular rows, but very irregularly. I am not sure that I can distinguish the median cord neuroblasts in *Doryphora*, though I believe that I have detected homologous structures. In my figure 72 I represented circular intersegmental patches in the median line between the lateral cords. Closer examination shows these to be clusters of cells of the same appearance and dimensions as the lateral-cord neuroblasts. They are very clearly brought out by Graber in his figures of *Hydrophilus* ('89, Figs. 40, 41, and 43, Pl. III) and are described as taking part in the formation of the posterior gangliomere of each ganglion. I doubt whether the large cells constituting the posterior gangliomere of *Periplaneta* in Miall and Denny's Fig. 43 ('86) to which Graber refers, are to be regarded as the equivalents of the median-cord clusters in *Doryphora* and *Hydrophilus*. *Periplaneta* very probably has in each segment only one median-cord neuroblast, which atrophies before the close of embryonic life, and the large cells in Miall and Denny's figure probably arise from the daughter-cells and are therefore merely large ganglionic elements.

Graber figures and describes ('89, p. 47, Pl. X, Fig. 130) a cross-section through an abdominal ganglion of a *Melolontha*

embryo in which he finds a neuroblast in either lateral cord and three symmetrically arranged cells of the same character in the median cord. Similiar median cells were seen in *Lucilia*. He refers to Korotneff's observations on *Gryllotalpa* and states that he has found the lateral "ganglionäre Grosszellen" in *Lina*. He is inclined to regard them as a widely occurring differentiation of the ectoderm.

In a subsequent paper ('90) Graber describes and figures a foliated condition of the ganglia in the nerve-cord of *Stenobothrus*. In Fig. 52 the neuroblasts are distinctly seen, and in one lateral cord five, in the other four pillars of cells may be distinguished. So far as the neuroblasts are concerned, he cannot be said to have added anything to Korotneff's account.

Nusbaum ('91), in a recent Polish paper on the development of *Meloë*, figures neuroblasts in the lateral cords. They are frequently represented in mitosis — the spindle-axes being in some cases perpendicular to the surface of the ganglia (Figs. 94, 95) while in others (Fig. 107) they are parallel to the surface, as in *Doryphora*. Such portions of the text as were translated for me contained nothing new on these structures.

Viallanes, in two recent papers ('90[a], '90[b]) on the structure of the nervous system in the embryo *Mantis*, comes to conclusions agreeing with my own, which were arrived at independently. His observations on the neuroblasts may be briefly summarized in his own words ('90[b], p. 293): "A l'origine le bourrelet primitif n'est qu'un simple épaississement de l'exoderme, c'est-à-dire une région de ce feuillet dont les cellules sont devenues columnaires et ont augmenté de volume. Bientôt ces cellules se multiplient et se divisent en deux couches, l'une superficielle (*dermatogène*), l'autre profonde (*gangliogène*). A une période plus ou moins tardive, suivant la région considérée, la couche des cellules dermatogènes se sépare de la couche des cellules gangliogènes et devient l'hypoderme. Les cellules gangliogènes en se multipliant donnent naissance aux cellules *ganglionnaires*."

Viallanes' figures do not show a regular arrangement of the cells budded forth from the neuroblasts, and he has not described the neuroblasts of the median cord, probably because

his attention was concentrated on the structure of the brain. He has observed the degeneration of the gangliogenic cells, or neuroblasts. In a late stage ('90[b], p. 301), he says "Ils montrent des signes évidents de décrépitude; beaucoup des cellules gangliogènes ont dejà disparu, les autres sont en voie d'atrophie."

Our knowledge of the median cord cannot be said to have made much advance since this structure was first described by Hatschek ('77). While all writers agree that it originally extends as an uninterrupted structure from the mouth to the anus, there is wide difference of opinion respecting the ultimate fate of its inter- and intraganglionic portions. Hatschek ('77), Tichomiroff ('82), and Korotneff ('85) maintain that the interganglionic portions remain attached to the integument when the nerve-cord is liberated and that they ultimately disappear. Ayers ('84) on the other hand holds that the whole median cord is liberated from the ectoderm, but does not affirm that the interganglionic portions form a constituent part of the ganglia.

Graber ('90) has very recently come to a conclusion which differs from the views hitherto advanced. With Ayers he holds that the interganglionic portions of the median cord are delaminated from the ectoderm along with the intraganglionic portions, but he goes further and claims (p. 103) that "das Zellenmaterial des interganglionalen Mittelstranges, zum Theil wenigstens mit den Ganglien vereinigt wird, oder mit anderen Worten, dass eine Vergrösserung des ganglionalen Mitteltheiles auf Kosten des interganglionalen erfolgt."

As will be inferred from the above descriptive paragraphs, I hold to Hatschek's view that the interganglionic portions of the median cord take no part in the formation of the ganglia but are drawn out from between the connections and constitute a portion of the sternal integument. Graber's researches on this portion of the nerve cord are limited to the Coleoptera and as the insects of this order certainly differ to some extent from the Orthoptera in the formation of the nervous system, I have no grounds for doubting the correctness of his observations. I believe, however, that Ayers' account of the median cord in *Œcanthus* is open to criticism. After

clearly implying that the median cord is set free from the ectoderm along its whole extent he remarks (p. 252): "Between the successive pairs of ganglia the median ingrowth atrophies, and at the time of the closure of the dorsal wall of the body there is seen between the connecting cords of two adjacent pairs of ganglia, a small triangular or cylindrical mass of cells, concerning the fate of which I am not absolutely certain. I believe, however, that they go to form a part of the internal skeleton. The chitinous rods in the thoracic region to which the muscles of the legs and wings are attached probably arise from the remnants of the median invagination, but in the abdominal region they may disappear entirely without giving rise to such structures." If I understand this passage correctly, Ayers implies that the chitinous rods are originally interganglionic portions of the median cord. But if this is the case, how can the median cord separate completely from the ectoderm unless we are to suppose that there is a reunion of the interganglionic portions with the integument to form the endoskeletal structures? The chitinous rods are directly continuous with the chitin of the integument so that until observations are forthcoming to show that portions of the integument can loosen and pass into the body-cavity and subsequently reunite with the integument, I must regard Ayers' account as inadequate.

I am still in some doubt as to the exact origin of the commissures. Grassi ('84), Ayers ('84), Heider ('89), and Graber ('90), all maintain that the commissural fibres arise from the median cord cells. *A priori*, there are no reasons why the daughter-cells of the median neuroblast should not send out processes to form Punktsubstanz and thus form a commissure. From the position of these cells, however, I regard it as highly improbable that anything but the posterior commissure could be formed in this way. The isolated Punktsubstanz masses in the Coleopteran median cord in Graber's and Heider's figures may arise from cells equivalent to the daughter-cells of the median neuroblasts of the Orthoptera. It is very improbable that the dermatoblastic cells which form the walls of the median cord in the region of the anterior commissure, and which I regard as

non-nervous, should take part in forming the fibres of that structure.

Regarding the origin of the neurilemmata in insects, there is still considerable doubt. The inadequacy and inconsistency of Nusbaum's observations on *Blatta* ('83) have been sufficiently pointed out by Eisig ('87) and Korotneff ('85). Nusbaum derived the median cord (which, by the way, he did not recognize as the median cord) from the entoderm, and compared it with the vertebrate chorda. So far his observations and conclusions were erroneous, but he derived the inner and outer neurilemma from the cells of this "chorda"—an observation which agrees essentially with my own.

Korotneff's view that the neurilemmata arise from migrant mesoderm cells has not been confirmed by recent writers, who are inclined to derive these envelopes from the ectoderm (Heider, '89 ; Graber, '90). Though I venture to say that my own observations are somewhat more definite than those hitherto published, I cannot regard them as in any way final.

2. *The Brain.*

In the following account of the *Xiphidium* brain, I shall use the nomenclature employed by Viallanes in his recent papers ('90[a], '90[b]), since his studies on the brain-development of *Mantis religiosa* agree very closely with my own. Before passing to a description of my sections I would refer the reader to the diagrammatic figure (VII) which represents the main points in the structure of the embryonic brain. Here it is seen that the ventral nerve-cord bifurcates just in front of the mandibular segment and passes on either side of the mouth, where it forms two successive pairs of ganglia. The posterior of these (*tc.*) is the *tritocerebrum*, or third brain segment. Its two halves are united by the *infraœsophageal commissure*, shown in the figure as a broad white band connecting the Punktsubstanz-masses of the ganglia. The anterior pair of swellings (*dc.*) constitute the *deutocerebrum*, or second brain segment. From this portion the antennæ are innervated. Further forward the deutocerebrum passes into a large paired

supraœsophageal mass, the *protocerebrum*, or first brain segment, which constitutes the greater portion of the brain. Each of its halves may be separated into three lobes; the first, or outermost lobe (*pc*¹ [*o.g.*]) forms the optic ganglion of the larva and imago, while the second and third lobes (*pc*², *pc* 3) ultimately form the bulk of the brain proper. The third lobe is united with the contralateral lobe by the broad *supraœsophageal commissure*. Such is the structure of the Orthopteran brain reduced to its simplest terms. It may now be considered a little more in detail.

Like the nerve-cord, of which it is simply a modified portion, the brain arises from neuroblastic cells. These first make their appearance in clusters (the spots seen on the procephalic lobes in Fig. 2). Later they form a single layer of proliferating centres continuous with and in every way comparable to the neuroblasts of the ventral nerve-cord. Like the latter they are covered externally by a layer of dermatoblastic cells.

That the deuto- and tritocerebral ganglia are strictly homodynamous with the ganglia of the nerve-cord is clearly shown in *Xiphidium*. In the first place these brain segments are directly continuous with the segments of the cord; second, they have at first the same size and shape as the latter, and third, they present on the average four neuroblasts in cross-section on either side. The suppression of the median cord in the deutocerebrum (if it be not drawn forward into the protocerebrum), is perhaps sufficiently explained by the presence of the stomodæal invagination. A partial suppression of the median cord in the tritocerebrum may be due to the same cause. The infraœsophageal commissure is perhaps the morphological equivalent of both the commissures of a ventral ganglion.

The early clustered condition of the neuroblasts is seen in Figs. 32–34 at *nb*. At the edges of these cross-sections a rounded mass of pale cells (*pc*¹ [*o.g.*]) is distinctly marked off from a more deeply stainable layer which encloses it on nearly all sides. This mass, the future optic ganglion (first protocerebral lobe), is delaminated from the ectoderm at a very early stage. The cells of the mass agree with the neuroblasts

in their slight affinity for stains; they differ in the more elongate shape of their nuclei and cytoplasm. The dark layer enclosing the optic ganglion on all except its innermost face is the *optic plate* and will give rise to the compound eye. Passing towards the median line in these sections (especially in Fig. 33) two other thickenings may be distinguished (*pc*^{2}, *pc*3) — the second and third protocerebral lobes.

The cross-section, Fig. 36, runs through the labrum of an older embryo (Stage F) and shows a considerable advance in the structure of the brain. The three protocerebral lobes are distinctly marked out. In the second and third, the neuroblasts have arranged themselves in a row and have budded forth strings of ganglion cells. In the first lobe (*pc*^{1} [*o.g.*]) no teloblastic arrangement is ever present. The cells are small and narrow and early assume a radial arrangement around the Punktsubstanz core at the base of the mass. The cells of the optic plate, which stand away from the surface of the ganglion, already show a tendency to differentiate in that they have become smaller and narrower than the dermatoblasts covering the two other lobes of the protocerebrum. At the juncture of the second with the third lobe, several large dermatoblastic cells are intercalated (*igl.*). They are continuous with the dermatoblasts covering the second protocerebral lobe. This intercalated mass is called by Viallanes the *bourrelet ectodermique intraganglionnaire*. I shall call it the intraganglionic thickening.

A still more advanced stage in the development of the brain is seen in Fig. 37 (Stage G). This section passes above the base of the labrum. Owing to the active proliferation of the neuroblasts, the mass of the protocerebrum is greatly augmented. The Punktsubstanz has made its appearance as a confluent mass.[1] The optic plate is much thickened and its small cells are about to arrange themselves to form the ommatidia. The intraganglionic thickening (*igl.*) presents an interesting appearance. The edge of the optic plate is united with the inner edge of the optic ganglia, but between it

[1] I have seen nothing to corroborate Cholodkowsky's view ('**91**) that there are at first three distinct and separate pairs of Punktsubstanz masses in the brain.

and the second protocerebral lobe there is a fissure (*w*) which extends in some distance. Examination of a number of successive sections and stages has convinced me that this fissure is not the result of artificial rupture during sectioning but that it is brought about by an infolding of the intraganglionic thickening. The shape and position of the involuted mass may be clearly seen from the surface in Stages H and I (see Figs. 8 and 9, *igl.*).

In the frontal section (Fig. 39) are shown the relations of the protocerebrum to the outer brain-segments and to the ventral cord. Only a small portion of the optic plate (*e*) is cut. Beneath it lies the optic ganglion (*pc*^{1} [*o.g.*]), the small cells of which contrast with the large cells of the brain proper. The second protocerebral lobe (*pc*^{2}) still contains many neuroblasts which are budding forth their last progeny. The older daughter-cells have already assumed an irregular arrangement. The brain is separated from the attenuated dermatoblastic, or integumental layer (*db.*) beneath which the outer neurilemma (*enl.*) is forming. The inner neurilemma (*inl.*) envelops the Punktsubstanz portions of the brain. The broad supraœsophageal commissure (*s.cm*) connects the third protocerebral lobes of the two sides. As shown in the figure, the deutocerebrum is distinctly præoral. At *an* is shown the point where the antennary nerve leaves the fibrous portion of this brain segment. Caudad to the deutocerebrum lies the tritocerebrum, a pair of somewhat smaller ganglia united by the infraœsophageal commissure. It is this segment which according to Viallanes innervates the labrum and the frontal ganglion. Besides the supra- and infraœsophageal commissures and the connectives which arise in the third protocerebral lobes and traverse the deuto- and tritocerebrum to pass into the mandibular ganglion and thence through the nerve-cord, I may call attention to two other masses of Punktsubstanz which lie in front of the supraœsophageal commissure. These are shown at *p.* in the figure. They appear to be connected by a small band running parallel to the robust supraœsophageal commissure. I did not succeed in finding these connected Punktsubstanz masses in all embryos of this stage, and as they were not

seen in later stages, their morphological value as indicating the presence of another segment in front of the protocerebrum is somewhat doubtful.

The three divisions of the protocerebrum may still be recognized in the transverse section (Fig. 40) of an embryo which has passed beyond Stage J. In the median line at *mc* lies a rounded and compact mass of cells which I regard as the præoral representative of the median-cord cell-masses of the ventral cord. A large cell, which I take to be a neuroblast, lies at the outer periphery of this median cell-mass at a younger stage. Structures at a corresponding position in the brains of other insects (*Hydrophilus*, *Musca*) and likewise comparable to the median-cord have been described by Heider ('89) and Graber ('89, p. 49). It is not improbable that the brain neurilemmata may have their origin near this median cerebral mass, just as the neurilemmata of the ventral cord probably arise from the non-ganglionic portions of the neural furrow.

Two other interesting structures are shown in Fig. 40: the intraganglionic thickening (*igl.*), now completely separated from the integument and lying as a deeply stainable mass wedged in between the optic ganglion and the second protocerebral lobe, and a peculiar pale thickening at the edge of the optic plate (*th*).

In later stages it is very difficult to locate the intraganglionic mass, so that I am unable to decide whether it atrophies or persists in a modified form as a portion of the brain. The researches of Viallanes on *Mantis* would seem to lend great probability to the former alternative.

The thickening at the lateral edge of the optic plate is constant in Stages G to J and somewhat later. It soon entirely disappears, without taking any part in the formation of the eye, so far as I have been able to observe. Does it represent an abortive ocellus?

The fully established optic nerve is shown in Fig. 41 (*o. n.*). In a much earlier stage it may be found as a delicate band of cells connecting the posterior edge of the optic ganglion with the optic plate (Fig. 38, *o. n.*). I agree with Viallanes, that it seems to arise from the ganglion and to grow outwards into the ommatidial layer; for there is from the first a sharply defined

intercepting membrane between the nerve and the plate, whereas the nerve passes without interruption into the ganglion. But I am led to lay little stress on these appearances by the researches of Watase ('90) and Parker ('90) on the adult ommatidia of a number of Arthropods. They have shown in a very convincing manner that each retinula-cell is the termination of an optic nerve fibre. The retinulæ are undoubtedly modified optic plate cells; and judging from recent observations on percipient cells in other forms (vertebrate eye, ear, olfactory nerves, v. Lenhossék's observations on *Lumbricus* ('92), etc.), we must suppose that the nerve fibres grow out from the retinula-cells, pass through the optic nerve and enter the ganglion. Such prolongations from all the retinulæ would be amply sufficient to form the optic nerve, although it is probable that some of its fibres are centrifugal prolongations from optic ganglion cells. It has been suggested that the optic nerve may be established at a very early stage, when the optic plate and optic ganglion are still closely applied to each other (*vide* Figs. 32–34), and that the nerve may not become visible till the two Anlagen separate with further development. But I do not think this is the case in *Xiphidium*. Sections like Fig. 36 show a distinct separation of the optic plate and ganglion in the region of the future optic nerve; and Viallanes has made an exactly similar observation on *Mantis*.

The sympathetic nervous system arises in part at least from the dorsal median wall of the œsophagus. At three separate points (Fig. 61) the ectoderm becomes thickened and its outer cells enlarge and assume the character of ganglion cells. The most anterior of these thickenings (*f. g.*) is the frontal ganglion. It arises just behind the base of the labrum. The two other thickenings which are placed further back (rg^1, rg^2) are the second and third visceral ganglia. I have not followed the development of the nerves which unite these ganglia and ramify from them.

Concerning the origin of the peripheral nervous system I have no positive data. In a few cases I have seen appearances which led me to believe that they arise as outgrowths from their respective ganglia.

The development of the brain of *Blatta germanica* and *Melanoplus femur-rubrum* agrees in all essential respects with the development of the *Xiphidium* brain. Certain Hemiptera, *e.g. Ranatra fusca*, conform very closely to the type of brain structure seen in these Orthoptera. I may mention in this connection that the brain of *Anurida maritima* shows the typical division into proto-, deuto- and tritocerebral segments with great distinctness. The last segment especially is remarkably distinct.

Until very recently the detailed study of the embryonic Hexapod brain has been limited to the Coleoptera and the results obtained have been naturally enough extended to include not only other insects but other Arthropods as well. The Coleoptera, however, are far from being primitive forms and the rôle which they play in contemporary embryological literature is largely attributable to the unusual technical advantages presented by their eggs. As far as development is concerned, the simpler brain of the Orthoptera and Ametabola in general offers many points of resemblance to the Crustacea and Myriopoda,[1] whereas the brain of the Metabola, like so many other points in their organization bears witness to a considerable amount of modification. It is therefore more consistent with our general views of phylogeny to reduce the Coleopteran brain to the Orthopteran type than to proceed *vice versâ*.

We owe the most important contributions to the subject of Orthopteran brain development to Viallanes. After a decade of study devoted to the histological structure of the adult Arthropod brain he has selected *Mantis* as a subject for embryological investigation. His previous careful study of the adult brain of other Orthoptera (*Œdipoda coerulescens* and *Caloptenus italicus*, '87[b]) has enabled him to avoid the confusion with which the inexperienced investigator is overwhelmed when attempting to follow the rapidly increasing complication of neural structures. With his usual skill and patience he has traced the development not only of the main structural features but of many details, so that we have a well-established point

[1] See the papers of St. Remy ('90) and Viallanes ('87[a], '87[b]).

of departure for further comparative studies. So far as my own observations are concerned I am able to corroborate Viallanes' results on nearly all important points. I must state, however, that I have not followed the development into such detail.

In the light of these researches a reconsideration of the Coleopteran brain must be undertaken. Patten's description of the *Acilius* brain ('88) and my description of the brain of *Doryphora* ('89) need revision and alteration. We described the organ (see my Fig. 72, Pl. XIX) as consisting of three segments, each of which was subdivided into a brain portion, continuous with the ganglia of the ventral cord, an optic ganglion portion and an optic plate portion. Between the third brain and the mandibular segment, a segment was found which I designated as intercalary. This segment is also clearly seen in some of Patten's figures (Figs. 2 and 2^a $s4$ Pl. VII). Thus according to our account there were four premandibular segments or seven segments in the entire head. Our results were obtained almost exclusively from surface views — by itself a defective method. But greater error was incurred, it seems to me, in ascribing segmental values to the various prominences of the optic ganglion and optic plate.

In order to bring our observations into harmony with the results obtained from a study of the Orthopteran brain, our figures must be interpreted in a very different way from that in which we chose to interpret them. Our first brain-segment is probably no segment at all, but merely a slight elevation often seen near the median line at the extreme anterior end of the germ-band. Our second, third and intercalary segments are equivalent to Viallanes' third protocerebral lobe, deutocerebrum and tritocerebrum. The three divisions of the optic ganglion are not parts of three segments, but the whole structure belongs to the protocerebrum, of which it forms the first lobe. In the same way we cannot regard the optic plate as trisegmental since it has no connection with the deuto- and tritocerebrum but only with the optic ganglion. It follows that the ocelli of Coleoptera are not originally formed on different segments as Patten would have us believe, but

belong to one segment — the protocerebrum. That such is the case I am convinced from a study of the eyes in embryos of *Dytiscus verticalis*, a form closely related to *Acilius*.

As will be seen in the profile view Fig. 8, the optic ganglion and optic plate of *Xiphidium* are at first folded back so as to lie along side the deuto- and tritocerebrum. The antennal furrow runs forward, separating the optic ganglion from the brain but stops when it reaches the protocerebrum. The value of this furrow as completely separating the second and third brain-segments from the optic ganglion was overlooked by Patten and myself: hence our false interpretation of the structures lying laterad to it.

I believe that I am justified in putting this new interpretation on the Coleopteran brain, because it harmonizes with Heider's careful study of *Hydrophilus* ('89). He has failed to find indications of segmental constrictions in the optic plate and optic ganglion and his figure 4 *A. B.* at p. 37 agrees closely with Viallanes' description of the *Mantis* brain. It should be observed that the embryos of *Hydrophilus* are much larger than those of *Acilius* and *Doryphora* and therefore much more favorable for surface study. On the other hand it may be urged that Heider evidently did not employ so good a method of surface preparation as Patten.

The distinct invagination associated with the formation of the optic ganglion in Coleoptera and described by Patten, Heider and myself, is probably homologous, as Viallanes suggests, with the intraganglionic thickening of the Orthopteran embryo. This structure in *Mantis*, and probably also in *Xiphidium*, takes no part in the formation of the optic ganglion, which arises — at least in great part — by delamination as in the Crustacea (see Parker ('90)). Only the outer or lateral portion of the optic plate becomes the compound eye, so that in a later stage the intraganglionic thickening is separated from the edge of the eye by a considerable space. The thickening then lies just laterad to the antennal furrow as shown in Fig. 8. Whether or not the invagination in the Coleoptera really plays any part in forming ganglionic tissue as has been claimed, must be decided by renewed investigations.

Concerning the researches of Cholodkowsky on the brain of *Blatta germanica*, I must say a few words, since I have described the brain and nerve-cord of this form as agreeing in all essential respects with those of *Xiphidium*. Cholodkowsky lays great stress on the existence of three distinct pairs of Punktsubstanz masses in the supraœsophageal ganglion as indicating the presence of three segments. When we come to examine his figures we find that he takes a very unusual view of brain-segmentation, for the three pairs of Punktsubstanz masses are seen to belong (Fig. 46 Pl. IV; Fig. 67 Pl. VI) to the protocerebrum and correspond to the centres of its three lobes. He did not distinguish the deuto- and tritocerebral segments! Such of his remarks on the development of the brain and ventral nerve-cord as are at all comprehensible show similar glaring defects in observation. Thus he has failed to detect the small dermatoblastic cells which from the first cover the brain and nerve-cord. He asserts that these organs are at first naked and are only subsequently covered by an overgrowth of the integument from the sides of the body. The antennal and neural furrows do not play the part in development that he ascribes to them. The last abdominal ganglion of the mature embryo does not consist of four but of three fused ganglia; the fusion of the second and third abdominal ganglia was completely overlooked.

3. *General Remarks on the Nervous System.*

The nervous system of Arthropods is by common consent derived from the nervous system of annelid-like forms, and it is to this group that we naturally turn in seeking an explanation for certain structures in the Hexapod brain and nerve-cord.

In a brief preliminary paper Patten ('88) made the statement, that "the ventral cord and brain of Arthropods is at first composed entirely of minute sense-organs, which in scorpions have the same structure as the segmental ones at the base of the legs." This would seem to indicate that the Arthropod nervous system can be traced back to the condition seen in Polychæta — *Lopadorhynchus*, according to Kleinenberg ('86) — where both brain and nerve-cord arise in connection with and

ultimately supplant certain larval sense-organs. So far, however, as the Hexapoda are concerned, Patten's statement is, to say the least, inapposite, since, as I have pointed out, both brain and nerve-cord arise from peculiar ectodermal cells — the neuroblasts — which under no circumstances can be regarded as primitively sensory. They are simply generalized cells, like the teloblasts of worms and the meristem of plants.

The development of the nerve-cord in the Hirudinea and Oligochæta agrees more closely with the conditions seen in insects. As Whitman has shown for *Clepsine* ('87), and E. B. Wilson for *Lumbricus* ('89), the nerve-cord is proliferated forward from a pair of neuroteloblasts situated at the posterior end of the germ-band. Hence, in these worms, the whole of the nerve-cord is condensed, as it were, into two huge mother-cells, whereas in the Insecta it is condensed into a single layer of huge cells. There are reasons, however, for believing that this layer is, in part at least, derived from a few large cells situated just in front of the anus, and therefore corresponding to the Annelid neuroteloblasts.[1] That there are only two rows of these cells in Annelids, while there are eight in insects, can form no very serious objection to their homology, as I pointed out in my preliminary note ('90c).

Certain conditions in the Crustacea also lend probability to the view that the Hexapod neuroblasts may be budded forth from a præ-anal row of teloblasts.[2] Patten ('90) has pointed out in *Cymothoa* a row of proliferating cells which form ectoderm, and Nusbaum ('91) has described a very similar condition in *Ligia*. I have observed the same phenomenon in *Porcellio*, and believe it to be of general occurrence throughout the Isopoda. The cells are budded forth so as to form regular transverse and longitudinal rows. Reichenbach describes and

[1] What I have called the neuroblasts in insects therefore correspond to the "neural cell-rows" in Annelids (the cells *np. c.* in E. B. Wilson's Fig. 59, Pl. XIX, and the cells *nc.* in Whitman's Figs. 9 and 11, Pl. V).

[2] The neuroblasts of the last row (*th.*, Fig. 56, Pl. VI) in the nerve-cord of *Xiphidium* are always distinctly larger and clearer than the neuroblasts of the remainder of the cord. They may be true neuroteloblasts and give rise to the neuroblasts, but as I have never found unmistakable caryokinetic figures in them, I am still in doubt as to their homology with the neuroteloblasts of worms.

figures a very similar budding-zone of ectoderm cells in the Decapod *Astacus* ('86). That a portion of the ectoderm cells thus budded forth in these forms goes to form the nervous system, admits, it seems to me, of very little doubt. The clustered condition of the neuroblasts in the young germ-band of *Xiphidium* may be due to the rapidity with which the germ-band grows in length and breadth; the original regular arrangement of the cells being thereby disturbed and not re-established till a somewhat later stage.

Although I have maintained a phylogenetic connection of the insect neuroblasts with the neural cell-rows of Annelids, I admit that they may be regarded from an entirely different standpoint, *viz.* as having arisen independently in insects by a process of precocious segregation; but it should be noted in this connection that it is just the oldest Pterygota, the Orthoptera, which show this segregation most clearly, while in the more recent forms (Coleoptera, *etc.*) the process is more obscure.

A comparison of the histogenesis of the insectean with the histogenesis of the vertebrate nervous system brings out some interesting analogies. The neuroblasts may be compared with His' Keimzellen which divide by caryokinesis to form his neuroblasts. These are directly converted into ganglion-cells by sending out axis-cylinder processes. They correspond, therefore, to the daughter-cells of the insect neuroblasts, which are likewise converted into ganglion-cells. The "Keimzellen" of vertebrates differentiate close to the central canal, which is, of course, morphologically the outer surface of the cord, just as the Arthropod neuroblasts lie at the surface of the cord. In both groups the daughter-cells appear to be budded off in the same direction morphologically; though in vertebrates the ganglion-cells migrate, while in insects they are pushed inwards by their newly proliferated sister-cells. The early separation of the neural ectoderm in vertebrates into Keimzellen and sustentacular tissue (spongioblasts of His) is paralleled in insects by the precocious splitting of the same germ-layer into neuroblasts and dermatoblasts, the latter giving rise to supporting tissue in the form of neurilemmata.

The majority of authors hold that the Arthropod brain is either wholly or in part homologous with the Annelid brain. Patten ('90) alone takes the view that the Annelid prostomium is absent in Arthropods, and that the brain of the latter is formed by the moving forward of three segments which are postoral in the Annelids. Apart from the fact that we have as yet no means of deciding whether what we call the first segment of the Arthropod head (protocerebrum) is really a single segment or a complex of several, it is extremely improbable that so highly important a structure as the Annelid brain should have completely disappeared in the Arthropods. So great is the resemblance between the Arthropods and Annelids in all the more important morphological features and even in the detailed structure of the ventral nerve-cord, that the complete elimination of the brain certainly makes strong demands on one's credulity.

Will ('88) goes to the opposite extreme and regards the præoral portion of the Arthropod brain as the exact homologue of the Annelid brain. He goes so far as to call the procephalic lobes of *Aphis* the "Scheitelplatte." He finds that they lie at the pole of the egg opposite the blastopore, or rather what he takes to be the blastopore, and that they arise independently of the nerve-cord. Now the "Scheitelplatte" of *Aphis* must include at least the proto- and deutocerebral segments — probably also the tritocerebrum. The deutocerebrum in all the Orthoptera which I have examined is provided with a pair of true mesodermic somites and with a pair of appendages, the antennæ. Each mesodermal somite sends a hollow diverticulum into an antenna, which is thus shown to be homodynamous with the other appendages of the embryo. The tritocerebral segment also contains a pair of abortive somites and in *Anurida maritima*, as I have lately ascertained, bears a pair of minute but distinct appendages (see Fig. VI, *tc. ap.*). Viallanes ('87[a]) and St. Remy ('90) have found that the second pair of antennæ in Crustacea belong to the tritocerebral segment. These facts go to show that the deuto- and tritocerebral segments are homodynamous with the postoral segments and, as the "Scheitelplatte" of *Aphis* must extend at least as far back as the

tritocerebral segment, it cannot be homologized with the Annelid Scheitelplatte, a structure which is not segmented.

A view midway between Will's and Patten's probably accords best with the facts at our disposal. The Arthropod protocerebrum probably represents the Annelid supraœsophageal ganglion, while the deuto- and tritocerebral segments, originally postoral, have moved forward to join the primitive brain. This is essentially Lankester's view ('81), according to which in Arthropods "the præœsophageal ganglion is a syncerebrum consisting of the archicerebrum and of the ganglion masses appropriate to the first and second pair of appendages which were originally postoral, but have assumed a præoral position whilst carrying their ganglion-masses up to the archicerebrum to fuse with it."

In comparing the Arthropod with the Annelid brain much stress has been laid on the fact so clearly brought out by Kleinenberg ('86) — that the Annelid supraœsophageal ganglion originates independently of the ventral nerve-cord. Several investigators — Balfour ('80), Schimkewitch ('87), Will ('88) and others — have fancied that they could detect a similar ontogenetic discontinuity of the brain and nerve-cord in Arthropods. But more recent observations all tend to prove that there is a direct continuity of the central nervous system from the time when the ganglia first make their appearance. So far as the insects are concerned I may note that Will's conclusions were based on the defective surface observation of a form (*Aphis*) ill adapted to the study of the central nervous system.[1]

Even granting that the Annelid brain arises independently of the nerve-cord — and this is not yet settled — at least so far as the Oligochæta are concerned (see E. B. Wilson, '89) — Lankester's view of the Arthropod brain is in no way invalidated. The line of separation corresponding to the Annelid prototroch must fall in front of the deutocerebral segment, since it has been shown that this segment in some insects contains a pair of well-

[1] Little value can be attached to Cholodkowsky's assertion that in *Blatta* the supraœsophageal ganglion originates independently of the nerve-cord, since he has failed to see the deuto- and tritocerebral segments which are quite as well developed in *Blatta* as in other Orthoptera.

developed mesodermic somites. When we stop to consider the intimate union of the proto- and deutocerebral ganglia from the time of their first appearance, we need entertain little hope of finding traces of a separation which existed, if indeed it existed at all, in a very remotely ancestral period.

VI. The Development of the Reproductive Organs in the Insecta.

1. *The Gonads.*

The following account of the development of the sexual organs is based almost exclusively on *Xiphidium*. Some attention was devoted to the study of *Blatta*, but this form proved to be so much less satisfactory and to depart so little from the *Xiphidium* type, that it was abandoned.

Before passing to a description of the sexual organs and their ducts, it will be necessary briefly to consider the mesodermal somites, since the history of the organs under consideration is intimately bound up with the history of the middle germ-layer. The mesoderm of *Xiphidium*, like that of other insects, is coextensive with the blastopore and hence reaches from the region of the definitive mouth to the region of the definitive anus. At first a continuous cell-layer, it soon splits up into segmental masses as metamerism sets in. These are further divided in the median ventral line so that each segment has a pair of mesoderm blocks. Each of these acquires a cavity and the somites are established.

The appendages are from the time of their first appearance intimately connected with the somites, since each of the latter sends a hollow diverticulum into the appendage of the corresponding half of the body. All the somites are fully formed when the embryo has reached Stage F. There are then eighteen pairs in all. The most anterior pair occupies the deutocerebral segment and sends hollow diverticula into the antennæ. The walls of these somites are much thinner than those of succeeding pairs and, curiously enough, persist much longer. The pair in the tritocerebral segment is very small

and indistinct and disappears very early. Each of the succeeding segments, with the exception of the eleventh abdominal has a distinct pair of somites. In the last abdominal, mesoderm is present, but I have been unable to find a trace of a true cœlomic cavity.[1]

The youngest embryo in which I was able to detect reproductive cells had almost reached Stage F. In still earlier stages careful scrutiny failed to reveal any differentiation of the mesoderm cells. These cells, it is true, vary considerably in size and appearance but I have found it impossible to fix on any one set of elements which might be brought into connection with the germ-cells of older embryos. It is not, therefore, till the somites are established as distinct sacs that unmistakable primitive germ-cells make their appearance. In frontal sections of embryos in Stage F (Fig. 52 *gd* 1, *gd* 3), the primitive germ-cells are seen to lie in the inner wall of the somite. They are considerably larger than any of the surrounding mesoderm cells, and much paler. The chromatin of their nuclei is arranged in a more delicate skein. Like the neuroblasts they stain very deeply in picric acid. Normally, they occur only in the first to the sixth abdominal segments, each cluster being confined to the inner wall of a somite. The reproductive organs of *Xiphidium* are therefore truly metameric in their origin. There is nothing to show that they arise from vitellophags which have migrated into the somitic wall ; nor can they arise from the entoderm, since they are differentiated before the entoderm-bands have reached the basal abdominal segments in their growth backward from the oral and forward from the anal formative centre. I conclude, therefore, that the primitive germ-cells are enlarged and modified mesoderm-cells. In explanation of Fig. 52 it may be noted that the plane of section is somewhat oblique so that it

[1] I mention this because Graber ('**90**) has recently described a cœlomic cavity in the anal segment of *Hydrophilus* (Fig. 29, p. 62). Cholodkowsky also describes and figures ('**91**, Fig. 49 Pl. IV) such a cavity in the eleventh abdominal segment of *Blatta*. Every little slit in the mesoderm is not a cœlomic cavity, and the figures referred to show only small spaces between the mesoderm cells of the telson. This may have been produced artificially, for aught the figures show to the contrary.

strikes only the lowermost germ-cell of the cluster in the third abdominal segment (*gd* 3) and in the fourth segment passes completely under the cluster. Even at this time certain mesoderm-cells, the future epithelial elements (*ep.*) begin to flatten out and apply themselves to the surfaces of the germ-cells.

The exact relations of the primitive germ-cells to the walls of the somite are readily seen in transverse section (Fig. 53). The inner face of the triangular somite is applied to the surface of the yolk and besides giving rise to the germ-cells will ultimately form the splanchnic, or visceral layer. The remainder of the cœlomic wall is somatic, or parietal, and is converted into fat-body and musculature. The heart arises where the outer edge of the splanchnic passes into the somatic layer. In the section figured the entoderm is still wanting on the left side, whereas on the right side a single cell (*en*) of the right posterior band has already reached the segment. Similar inequalities in the rate of growth of the entoderm-bands are by no means infrequent.

In this stage some of the primitive germ-cells show a tendency to leave the wall of the somite and to drop into the cœlomic cavity. This is distinctly seen in Fig. 53. These cells sometimes enlarge considerably, become vacuolated and take on the appearance of young ova. A cell of this kind, nearly filling the cœlomic cavity, is shown in Fig. 55. I do not believe that the cells are loosened from the cœlomic wall during the process of sectioning.

Although the clusters of germ-cells normally occur only in the first to the sixth abdominal segments, in one somewhat older embryo (Stage G) a well developed pair of clusters was found in the tenth segment. One of these is shown in sagittal section in Fig. 56 (*gd* 10). It resembles the normal clusters in every particular. The same section shows the diverticulum of the tenth somite (*m. d.*). In the next section laterad to the one figured, the hollow tip of the diverticulum is seen to terminate in the right appendage of the segment. A similar relation of the cœlomic diverticula to the appendages obtains in all the abdominal segments in front of the tenth.

The primitive germ-cells, which at first occupy only a limited portion of the splanchnic wall, increase in number during a stage midway between F and G. Beautiful caryokinetic figures may then be found in some specimens — showing that the primitive germ-cells themselves proliferate. New germ-cells may be added to the clusters by a differentiation of elements in the splanchnic wall but I have seen nothing to convince me that this occurs. The epithelial cells become much flattened and stain more deeply so that they stand out distinctly among the pale rounded germ-cells which they invest. The inner wall of the somite soon becomes too small to contain all the rapidly accumulating cells and is forced to send out a solid diverticulum. This is directed anteriorly, and in a little later stage fuses with the wall of the antecedent somite. This fusion is probably preceded by the shortening of the embryo which takes place during a stage immediately succeeding Stage F. The result of the fusion is the formation of a continuous strand of germ-cells with their accompanying epithelial cells. For some time the typical hexa-metameric arrangement is still visible in the strand, but later the whole mass shortens very decidedly to form the definitive ovary and testis and all traces of metamerism are lost. In the present paper I shall not follow the development of these organs further, but will pass on to a description of the sexual ducts. This will enable me to supplement the recent work of Heymons ('91) who has given us an extended and valuable account of the development of the sexual organs in *Blatta*, but has contributed only a few observations on the development of the ducts.

2. *The Male Ducts.*

The sexual ducts like the germ-cells are modified portions of the mesodermal somites. While considering the exceptional embryo in Fig. 56, attention was directed to the diverticulum (*m. d.*) of the tenth abdominal somite. This diverticulum, which is quite normal, is destined to form the terminal portion of the deferent duct (spermaduct) and the seminal vesicle of the adult insect. At the base of the divert-

iculum a constriction is formed which converts the proximal portion into a thin cord but leaves the distal end expanded as a hollow sac, which I shall call the terminal ampulla. The remainder of the deferent duct — *viz.* the portion extending from the sexual gland in the sixth to the anterior end of the tenth segment is formed by a cord-like thickening in the splanchnic wall of the three intervening somites. Anteriorly the cells of the duct pass into the epithelium enveloping the germ-cells. There is no lumen in the duct proper except towards its end where it widens into the terminal ampulla. Thus only the appendage diverticula of the tenth segment go to form the ends of the ducts ; in all the other abdominal segments the diverticula break down and disappear, together with their respective appendages, before the embryo reaches Stage H. In the thoracic, oral and antennary segments, however, the diverticula are converted into the muscles of the persistent appendages.

The further history of the male ducts is readily followed in partially stained embryos mounted *in toto*. Sex is determined, so far as I have been able to make out, during or soon after katatrepsis, at which time the appendages of the second to the seventh abdominal segments disappear. Fig. 42 represents the caudal end of an embryo just after katatrepsis (Stage J). Appendages still persist on the eighth to eleventh segments while the pleuropodia, not seen in the figure, have begun to degenerate. The testes (*ts.*) and the spermaducts (*m. d.*) are represented in blue. The former have shortened considerably and moved caudad so that they now lie in the sixth and seventh segments. The long terminal threads run forwards from the anterior ends of the testes, while the spermaducts run backwards and end in the terminal ampullæ (*ta. m.*) which still fit into the cavities of the tenth pair of abdominal appendages (*ap.* 10). A section through these appendages is seen in Fig. 57, showing very clearly the connection of the ampullæ (*ta. m.*) with the ducts (*m. d.*). In front of this section the ducts have no distinct lumen.

In Fig. 43, taken from a slightly older embryo, the appendages of the eighth segment have completely disappeared,

while those of the tenth have grown smaller and approached the median ventral line. They have, in fact, grown too small to contain the ampullæ which are drawn away from them and lie between the ninth and tenth segments a little in front of the abortive appendages. At the same time the inner faces of the ampullæ have become flattened and applied to each other in the median line. Each of these sacs has a pointed tip directed caudad. The more arcuate course described by the ducts in this stage is undoubtedly due to the mutual approximation of their terminal ampullæ. The appendages of the eleventh segment, the cerci (*cc.* [ap^{11}]), which in the preceding stage were rounded like the other abdominal appendages, have become oval.

A more advanced embryo is represented in Fig. 44 (somewhat younger than Stage K). The appendages of the tenth segment (ap^{10}) have almost completely disappeared. Those of the ninth segment, the future styli (ap^{9}) have lengthened and now point outwards and forwards. The cerci have grown more pointed. The terminal ampullæ lie completely in the ninth segment, having shifted their position headwards. The movement takes place in such a way that what were the posterior faces of the ampullæ in the younger stage (Fig. 43) are applied to each other, while the pointed tips are directed forwards. The ducts are thereby rendered still more arcuate towards their terminations. An intermediate stage in this singular movement is shown in Fig. 45, where only small portions of the posterior faces of the ampullæ are as yet applied to each other. Comparison of Figs. 43, 44 and 45 shows that the pointed tips remain united and move forward while the surfaces of mutual contact are being shifted. Finally in Fig. 46, which represents the abdominal end of an embryo ready to hatch, we see that the terminal ampullæ have increased considerably in size at the expense of the thickness of their walls. They have also lengthened, and brought still more of their surfaces in contact in the median line. The pointed tips of the ampullæ extend into the eighth segment. It may also be noted that the points where the spermaducts meet the ampullæ have moved forward. The appendages of the tenth segment

have long since disappeared and the pleuropodia have lost their organic connection with the embryo, so that only two pairs of abdominal appendages persist, the stylets (*st.* [*ap* 9]) and the cerci (*cc.* [*ap* 11]), both provided with setæ. The pointed fusiform cerci are now folded back so as to bring their insertions on the anal segment into view.

Beyond this stage the development of the male ducts was not followed in *Xiphidium ensiferum*, but several larval stages of an allied species, *X. fasciatum*, were studied for the purpose of connecting the embryonic with the adult condition.

It will be noticed that in *Xiphidium ensiferum* there exists at the time of hatching no external opening to the spermaducts; the ampullæ are completely closed sacs applied to the ventral hypodermis of the ninth abdominal segment, and the ducts connecting them with the testes have no lumen. In the *X. fasciatum* larva 10 mm. long the ejaculatory duct has made its appearance as an unpaired invagination of the hypodermis in the median line between the ninth and tenth segments. Fig. 47 shows the sexual organs of such a larva seen from within, the ventral scutes of the tenth and anal segments having been entirely removed. The prominent terminal ampullæ, which become the seminal vesicles of the adult, are considerably enlarged and their walls have increased in thickness. The short spermaducts, now provided with a small lumen, run from the under surface of the sacs to the prominent testes. Only the outer opening of the invagination which is to form the ejaculatory duct is seen at *m.o.* It runs forward as a flattened chitin-lined depression beneath the seminal vesicles. Sagittal sections show that there is as yet no communication between the lumina of the mesodermal and ectodermal portions ; it is not till a later stage that such a communication is established.

3. *The Female Ducts.*

The oviducts, like the vasa deferentia, are derived from a pair of cœlomic appendage-diverticula, but in the female the diverticula belong to the seventh abdominal segment. The diverticula of the female embryo also become constricted

proximally and end in terminal ampullæ, which are from the first somewhat smaller and more elongate than the homodynamous structures of the male. The appendages to which the ampullæ belong are also less prominent than the tenth pair of appendages in the male. Examination of several series of cross-sections from embryos in Stage J — this being the stage in which the sexes differentiate — reveals the curious fact that in the female, besides the pair of ampullæ in the seventh, a pair is also retained in the tenth segment. Figs. 58 and 59 represent two sections taken from such an embryo — the former passing through the tenth, the latter through the seventh abdominal segment. In Fig. 58, the two terminal ampullæ (*ta. m.*), and small portions of the ducts leading to them, are still preserved, but the cells and nuclei, especially in the ducts, are being broken down. The ampullæ soon share the same fate. In the seventh segment (Fig. 59, *f.d.*) the cavity of the diverticulum still opens into the cœlomic cavity of the same segment (*coc*). Its distal ampullar end is applied to the ectoderm where it bulges out to form the small seventh abdominal appendage (*ap*^{7}). The condition of the diverticulum after the constriction of its proximal portion is shown in Fig. 60, taken from a somewhat more advanced embryo. In this figure, the connection of the oviduct with the posterior end of the young ovary (*ov.*) is distinctly seen. The cells of the duct pass over into the epithelial cells of the ovary, just as the cells of the spermaducts become continuous with the testicular epithelium.

We may now turn to surface views of the female reproductive organs. The specimen represented in Fig. 48 is in the same stage as the male embryo represented in Fig. 42. Five consecutive pairs of abdominal appendages are still present (*ap*^{7}, *ap*^{11}). Of these, the ninth and eleventh pairs are very prominent, while the tenth pair has grown very small. The ovary (*ov.*) extends back to the seventh segment where it joins the oviduct. This ends in the terminal ampulla, which lies near the posterior edge of the segment in the seventh abdominal appendage. The terminal ampullæ of the tenth segment have not yet disappeared. They are represented in blue because I regard them as the homologues of the persistent male ampullæ.

In a more advanced embryo (Fig. 49, Stage K) the male ampullæ have disappeared completely, and the tenth pair of appendages, while growing smaller, have moved up to the inner posterior insertions of the ninth pair. The ampullæ have increased in size and have come to lie at right angles to the longitudinal axis of the embryo. This causes the oviducts to describe an arc. It is thus seen that the movement of the female ampullæ is essentially the same as that of the male, but considerably weaker. Traces of appendages on the seventh segment are still apparent.

From this stage we may pass to a brief consideration of the female embryo ready to hatch (Fig. 50). The ovaries (*ov.*) have now assumed their definitive characters. Although the pointed and flattened ampullæ have approached the median ventral line, they are still separated by a wide space. Even in this advanced stage slight thickenings of the integument over the posterior edges of the ampullæ may be taken to represent the remains of the seventh pair of abdominal appendages. The appendages of the tenth segment appear to have joined the inner bases of the ninth pair. I must say, however, that my observations on this pair of appendages are unsatisfactory, notwithstanding I have taken considerable pains to follow their history. The appendages of the eighth and ninth segments undoubtedly form the two anterior pairs of gonapophyses. The third pair has been described by Dewitz ('75) and others as arising from the inner bases of the second pair and is therefore supposed to belong to the ninth segment. I believe, however, that the tenth pair of embryonic appendages persists and moves forward to join the ninth pair, whence they grow out during early larval life as the third pair of gonapophyses. In the embryo the line separating the ninth and tenth segments is certainly very vague, especially on the ventral surface, so that the possibility of a fusion of the two pairs of appendages is by no means precluded. That this fusion should occur is certainly no more remarkable than the migration of the male ampullæ from the tenth to the ninth segment. Both of these forward movements may be in some way connected with the forward migration and fusion of the ganglia belonging to the eighth, ninth, and tenth segments (*cf.* Figs. 42–46, ag^{1-3}).

The female larva, like the male, has no external orifice to the sexual organs at the time of hatching. It is even more backward than the male, inasmuch as the terminal ampullæ of the oviducts have not yet met in the median ventral line. The first traces of a vagina were found in *Xiphidium fasciatum* larvæ about 10 mm. long (Fig. 51). Here the terminal ampullæ meet, but the surfaces of mutual contact are limited to the pointed tips. The vagina (*vg*) is a short and broad invagination of the hypodermis between the seventh and eighth segments. Its tip extends to the juncture of the terminal ampullæ. In a somewhat later stage the ampullæ open into each other and into the vagina. The three pairs of gonapophyses (*op*1–*op*3) are already assuming their definitive characters.

For an excellent résumé of the little work that has been done on the embryonic development of the sexual organs in insects I would refer the reader to Heymons' recent paper ('91). Here I shall consider only three contributions, — two of Heymons' ('90 and '91) which treat mainly of the sexual glands, and Nusbaum's paper on the development of the ducts ('84).

Although the results of my study of *Xiphidium*, so far as they go, agree in many respects with Heymons' account of *Blatta*, several not unimportant differences must be pointed out. The first difference relates to the stage in which the germ-cells make their appearance. Heymons ('91) claims that he can detect them before the somites are established, at a time, in fact, when the abdominal region of the embryo is still unsegmented. This would correspond to a stage in *Xiphidium* midway between *B* and *C*. In *Blatta* certain mesoderm-cells at this time enlarge and assume a clear and succulent appearance. There is apparently no definite relation between the position of these modified cells and the future segments, and even in a later stage when they become integral portions of the somite-wall, they are quite irregular in their distribution. Heymons regards them as largely dissepimental in position. In *Xiphidium*, which is, on the whole, a far more favorable form for the study of the sexual organs than *Blatta*, I was

unable to detect the presence of germ-cells till the somites were established (Stage a little younger than F). At this time they formed strictly metameric cell-clusters each of which was confined to the median portion of the splanchnic wall of its respective somite. These cells rarely, if ever, strayed into the dissepimental region during this stage. It is, of course, conceivable, that *Xiphidium* and *Blatta* may differ very considerably in respect to the point under consideration, but I suspect, nevertheless, that Heymons has mistaken the young vitellophags for sexual-cells, notwithstanding his assertion to the contrary. At any rate, to be complete, his figures should show the vitellophags, which are undoubtedly present in the stages he studied and which occupy the very location of his "sexual-cells" in his Figs. 2 and 3.

Another point on which we differ is the distribution of the germ-cells. According to Heymons they occur in *Blatta* in the second to seventh abdominal segments, whereas I find them in *Xiphidium* in the first to sixth. Heymons says emphatically: "Im ersten Abdominalsegment treten niemals Genitalzellen auf." But this is certainly an error, for in several *Blatta* embryos I find unmistakable germ-cells forming a pair of isolated clusters in the first abdominal segment. Here they also persist till a comparatively late stage when they are drawn into the second segment during the contraction of the sexual Anlage. The peculiarly modified pleuropodia in *Blatta* form so efficient a means for determining the exact position of the first abdominal segment and its somite in series of sections both longitudinal and transverse, that I feel confident of not being mistaken in this matter. I admit, however, that fewer germ-cells occur in the first than in the succeeding abdominal segments. According to Heymons comparatively few germ-cells occur in the seventh pair of abdominal somites. In these I have never seen traces of germ-cells in *Xiphidium* but I cannot, of course, assert that they never occur, especially as I have shown that germ-cells may be found even as far back as the tenth segment. It is interesting to note that Heymons, too, found germ-cells in some of the posterior abdominal segments in *Blatta*.

In his first paper ('90) Heymons made the following statement in regard to the genital ducts: "Von besonderer Wichtigkeit scheint mir nun die Thatsache zu sein, dass beim Männchen der ursprünglich angelegte Ausführungsgang nicht in seiner ganzen Länge zum Vas deferens wird, sondern dass sich sein distaler Abschnitt später wieder zurückbildet, ohne je functioniert zu haben. Der wirklich als Ausführungsgang dienende Endtheil des Vas deferens, welcher sich mit dem ectodermalen Ductus ejaculatorius verbindet, entsteht erst nachträglich an dem ursprünglich angelegten Ausführungsgang. Beim Weibchen dagegen bildet sich der ganze primitive Ausführungsgang zum Oviduct aus."

This is the very opposite of what I have found: in *Xiphidium* it is the male duct which at first occurs in both sexes in the tenth abdominal segment — whereas in the female the oviducts are an independent formation, the original male duct being soon broken down. In the female both pairs of ducts are established simultaneously since they are both cœlomic diverticula.[1]

In his more recent paper Heymons ('91) describes the genital ducts as terminating at the posterior edge of the seventh abdominal segment. As he mentions this fact before he comes to a description of the embryo with determinate sex, I assume that he regards these ducts as common to both sexes. What he saw was without doubt the pair of oviducts, not the deferent ducts. From personal observation I can state that the male ducts of *Blatta* end at first in terminal ampullæ enclosed by the appendages of the tenth abdominal segment just as in *Xiphidium*, whereas the female ducts terminate in much flattened ampullæ in the seventh segment. Whether or not a rudimental male duct is present in the tenth segment of the female *Blatta* embryo I have been unable to decide. Perhaps Heymons found something of this kind and while confounding the sex of the embryos he studied, was led to make the above quoted remark.

[1] For the sake of greater exactness, I may state that the anterior pair is, perhaps, formed a little sooner than the posterior pair, since the somites develop from before backwards.

Of the few observations which have been made on the development of the genital ducts in insects, Nusbaum's ('84) are the most important. Their agreement with Palmén's anatomical researches on Ephemerids ('84) has been regarded as sufficient warrant of their accuracy. Nusbaum studied the developing ducts in Mallophaga, Pediculidæ, Blattidæ, and Culicidæ and came to the conclusion that the testes and deferent ducts in the male and the ovaries and oviducts in the female are mesodermal, while the seminal vesicles, ejaculatory duct and accessory glands in the male, and the uterus, vagina and accessory glands in the female are ectodermal. He therefore draws the line between mesodermal and ectodermal portions at the juncture of the seminal vesicles and deferent ducts in the male and at the juncture of the oviducts and uterus in the female. This is at variance with my results, since I have found that the seminal vesicles and uterus are mesodermal. These structures are described by Nusbaum as if he had traced their derivation from the ectoderm step by step. Yet he seems not to have studied embryos but only larvæ, and it is during embryonic life that the uterus and seminal vesicles are formed.[1] Furthermore his investigations were carried on without the aid of sections. The differentiation of the uterus and seminal vesicles from the ectoderm is far from satisfactorily shown in his figures. I cannot therefore regard Nusbaum's work as contributing any evidence in favor of Palmén's view. Palmén concluded from a careful study of the Ephemeridæ that the genital ducts of insects originally

[1] This is Nusbaum's description ('84, p. 40): "Auf der Bauchseite des vierten, von hinten an, Abdominalsegmentes entstehen zwei paarige Hautepithelverdickungen die sich einander nähern um sich dann zu einem hufeisenförmigen unpaaren Körper zu vereinigen. Bevor aber noch die Vereinigung zu Stande kommt, lösen sich diese Keime von der Haut ab und verwachsen, wie gesagt, mit den Enden der noch soliden Vasa deferentia. . . . In dem vorderen Theile des soliden hufeisenförmigen Keimes entstehen zwei vordere geschlossene Höhlen; der mittlere Theil bleibt noch weiter solid, der hintere verlängert sich in zwei seitliche solide Auswüchse. Die zwei vorderen Höhlen verlängern sich nach vorn und differenziren sich in die zwei Vesiculæ seminales (innere Schläuche) des definitiven birnförmigen Körpers. Mit denselben communiciren die sich aushöhlenden Vasa deferentia." An essentially similar description is given by Nusbaum for the female (p. 41).

had paired openings on the surface of the body. This view, which I fully endorse, has a good basis of anatomical data; but Nusbaum has not shown that there is a double opening to the sexual organs or even a distinctly paired Anlage to the ectodermal portion of the sexual apparatus. According to his own figures the vagina and ejaculatory duct are unpaired from the first; the structures on which he laid stress as being paired ectodermal portions were nothing more nor less than the unmodified terminations of the mesodermal ducts — the terminal ampullæ.

4. *General Considerations.*

The foregoing account of the development of the sexual organs differs sufficiently from the accounts of other authors to justify a brief consideration of some general questions.

First in regard to the germ-cells. These arise as six metameric pairs of clusters in the splanchnic walls of the mesodermal somites. Since the single layer of cells forming the walls of each somite corresponds to the peritoneal epithelium of Annelids, Heymons' conclusion that the germ-cells of insects arise in essentially the same manner as the germ-cells of Annelids, is certainly well-founded. In both groups the germ-cells are local proliferations of the epithelium lining the body cavity. In Annelids the germ-cells lose their connection with the peritoneum and drop into the body cavity where they undergo maturation. I have called attention to a similar process in the *Xiphidium* embryo. Whether these germ-cells disintegrate or again attach themselves to the wall and become invested with epithelial cells, I must leave undecided. I am inclined to adopt the latter alternative, since I have found no traces of germ-cells in the cœlomic cavities in stages but little older than the one figured. (Fig. 53.) Heymons has observed in *Blatta* a similar migration of the germ-cells into the cœlomic cavity.

I have alluded to the fact that *Xiphidium* exhibits more pronounced metamerism in the early arrangement of the germ-cells than *Blatta*. Strictly speaking the germ-cells in the form studied by Heymons are not at all metameric since

they arise, if his account is correct, before metameres are established. It is only on the supposition that the germ-cells of *Blatta* are precociously segregated that their method of origin can be satisfactorily compared with the conditions seen in Annelids, for in this group the germ-cells are not differentiated — so far as I am aware — until after the somites have reached a considerable degree of development. Providing, therefore, that I have not overlooked the germ-cells in precœlomic stages, *Xiphidium* must be regarded as presenting more primitive conditions than *Blatta*.

In *Xiphidium* and *Blatta* six, and therefore more than half the total number of abdominal segments, produce germ-cells.[1] In one case I found well-developed clusters in the tenth segment, so that if we omit the eleventh or telson-segment, which is rudimental and hence cannot be expected to produce germ-cells, and if, moreover, Heymons is correct in stating that reproductive elements occur in the seventh, only two abdominal segments fail to produce germ-cells ! This consideration lends support to Heymons' suggestion that "ürsprünglich die Sexualzellen auch in den hinteren Segmenten des Abdomens noch in derselben typischen Weise auftraten." The resemblance of the insect-embryo to Annelids in which a great number of consecutive segments produce ova and spermatozoa, is very obvious. The high development of the appendages and their musculature in the thoracic and oral segments of insects perhaps sufficiently accounts for the complete elimination of the germ-cell clusters in these regions. It may also be noted that they are normally absent in the abdomen in the very segments which longest retain traces of *quondam* ambulatory appendages — viz. the eighth to the eleventh.

The indications of metamerism which are so transitory in the sexual Anlage of the Orthoptera would appear to be retained throughout life in some of the Thysanura. In *Iapyx*, according to Grassi ('89), the arrangement of the egg-tubes is "nettement métamérique," and his Fig. 44, Pl. IV, represents in either ovary seven egg-tubes, occurring in consecutive abdominal seg-

[1] In a single instance (Fig. 55, Pl. VI) what I took to be a sexual cell was found in one of the cœlomic cavities of the metathoracic segment !

ments, beginning with the first. The solid testes show no traces of metamerism. In the young *Lepisma*, according to the same authority, the egg-tubes are also segmental, there being five in either ovary, a pair in each of the five basal abdominal segments. In the adult this character is no longer noticeable. In certain species of *Lepismina*, there are six sacs in either testicle, united in pairs on either side. These also lie in the basal abdominal segments. "L'organisation segmentaire des ovaires chez *Iapyx* se répète chez *Machilis*. Cette répétition dans des formes très éloignés l'une de l'autre comme le sont précisément *Iapyx* et *Machilis*, tend à donner au fait une sérieuse valeur morphologique. Les tubes ovariques de *Machilis* sont au nombre de sept de chaque côté." In the latter form Oudemans ('87) also figures seven egg-tubes strung along the oviduct, but without a clearly marked metameric arrangement. In the male there are three pairs of testicular sacs.

In all these Thysanura the female, as might be expected, adheres more tenaciously than the male to the metameric scheme. It will also be observed that the number of egg-tubes (five to seven pairs) is about the same as the number of germ-cell clusters in embryo Orthoptera. The position of the organs is also identical, viz. in the first to seventh abdominal segments. We might conclude, therefore, that the sexual organs of the higher Thysanura represented an embryonic or arrested condition.

A difficulty is encountered, however, when we stop to ask the question: Is the individual egg-tube in such a form as *Machilis* homologous with an individual egg-tube in *Blatta* or any other Pterygote? So far as structure is concerned, this would appear to be the case. We should also say that each egg-tube of *Iapyx* or *Machilis* was a metameric unit. But the lowest number of egg-tubes in the *Blatta* ovary is sixteen, and as this is more than double the number of metameres which contribute germ-cells in the embryo, the egg-tube in this form cannot be regarded as a metameric unit. We must conclude, therefore, either that the individual egg-tubes are not homodynamous in the Pterygota and Apterygota, or that the ovaries in *Iapyx*, *Machilis*, etc., are not primitively metameric. The possibility of there being an acquired metamerism, or pseudometamerism

in these cases is suggested by Grassi: "Cette disposition qui est nettement métamérique préserve l'ovaire du danger d'être détérioré de quelque manière que ce soit. Le danger existe particulièrement quand les oeufs sont près d'arriver à maturité et provient de ce que, chez *Iapyx*, la differentiation segmentaire de la musculature et de la cuticule est avancée au point que les métamères ont acquis une grande independance de mouvement. Cette independance est beaucoup moindre chez *Campodea* et c'est pour cela que cet insecte n'offre pas la disposition indiqué plus haut." If this be an adequate explanation, the resemblance of the sexual organs in the Thysanura to those in the Orthoptera is due to secondary causes. At all events, this question must remain open till Thysanuran embryos can be studied.

The metameric mesodermal origin of the germ-cells in embryo Orthoptera is too much like the origin of the germ-cells in Annelids to be considered as secondary and I fully agree with Heymons ('91), and Korschelt and Heider ('92) in regarding the sexual organs of such forms as the Rhynchota (Aphidæ, Cicadidæ) and Diptera (*Chironomus*, *Cecidomyia*) as derived by a process of precocious segregation from metameric gonads like those of the Orthoptera. These exceptional forms frequently exhibit peculiar and aberrant features (parthenogenesis, pædogenesis) like the Crustacea which have a similar precocious segregation of germ-cells (*e.g.* *Moina*, according to Grobben, '79).

The genital ducts of the insect embryo are not so readily reduced to the Annelid type. Many authorities, it is true, have regarded them as modified nephridia but apart from their paired mesodermal origin and tubular structure there was very little to support such a view.[1] But now the prevailing view receives fresh support from the fact that the ducts in both sexes arise as hollow diverticula of the cœlom. Though temporarily obliterated the lumen of the duct is very probably a persisting remnant of the cœlomic cavity. This is certainly

[1] My statement in a former paper ('89) that the genital ducts might arise from tracheal involutions is erroneous. What I saw and figured (Fig. 80, Pl. XIX) was a section through the terminal ampullæ of the deferent ducts, and not as I supposed, through their orifices.

the case with the cavities of the terminal ampullæ which are never obliterated.

In seeking for some clue to the true nature of the cœlomic diverticula one naturally turns to *Peripatus*. Unfortunately the two accounts of the development of the nephridia and genital ducts in this curious Arthropod — the one by v. Kennel ('85 and '88), the other by Sedgwick ('85 and '88) — contradict each other in many particulars. Both authors, however, agree in deriving the mesodermal portion of the nephridium from hollow diverticula of the somites (similar to those seen in the Orthoptera, in that they extend into the appendages!), and both agree in regarding the sexual ducts as modified nephridia. But Sedgwick derives the nephridium from the portion of the diverticulum located in the appendage, while Kennel derives it from the inner lower angle at the base of the somite. According to Kennel only the funnel-portion arises in this way, the long duct being formed by a tubular invagination of the ectoderm. On the other hand, Sedgwick derives the funnel and the greater portion of the duct from the mesoderm and believes that only a very small portion of the duct arises by invagination from the ectoderm. These differences apply, of course, to the sexual ducts as well. According to v. Kennel's account not only their unpaired terminal portion (opening in his form on the antepenultimate segment) but also the deferent ducts and uteri are ectodermal; only a short piece, corresponding to the nephridial funnel, and uniting the uteri to the ovaries, and the deferent ducts to the testes, has a mesodermal origin. According to Sedgwick the dorsomedian portion of the cœlom persists in the segments caudad to the fifteenth and is constricted off from the remainder of the somite. The dissepiments are broken down between the adjacent abstricted portions of the somites, so that a hollow tube is formed on either side. These tubes receive the germ-cells from the entoderm and form the sexual glands.[1] In the segment bearing the anal papillæ

[1] Sedgwick claims that the germ-cells originate in the entoderm and later on migrate into the cœlomic wall. In this particular I prefer to adopt v. Kennel's account, according to which the germ-cells have a mesodermal origin, since it accords better with the facts of Annelid development and with my own observations.

(which in all probability are reduced ambulatory appendages) a complete separation of the cœlom into a lateral (diverticular) and a dorsomedian (genital) portion does not take place, so that the two cavities remain confluent. The portion of the cœlomic wall surrounding the proximal cavity joins the sexual-gland while the diverticular (nephridial) portion acquires an external opening, "which, however, is much nearer the middle line than in the case of the anterior somites, and, indeed, may be described as being common with that of the opposite side. However this may be, the two openings soon become definitely united to form a single opening, while the tubes themselves persist as the generative ducts. Whether any large portion of the latter are ectodermal in origin, that is to say, derived from a growth of the lips of the opening at its first appearance, it is impossible to say."

If we accept Sedgwick's account it is easy to reduce the genital ducts of insects to the type seen in *Peripatus* and consequently to Annelid nephridia. In the first place, everything goes to show that the appendage diverticula of the cœlom are homologous both in *Peripatus* and Orthoptera. In both cases the oviducts and deferent ducts arise from these diverticula by partial constriction. Just as the ducts of *Peripatus* run into the cavities of the anal papillæ, so the sexual ducts of *Blatta* and *Xiphidium* run into the rudimental abdominal appendages. In both cases there are terminal ampullæ, for as such I venture to regard the slight distal widening of the cœlomic diverticula in Sedgwick's Figs. 42 and 44. A comparison of these figures with my Figs. 56 and 59 will show the close resemblance between insects and *Peripatus* better than paragraphs of description.

As the exact limits of the ectodermal portions of the ducts of *Peripatus* have not been clearly ascertained, further comparison with the Insects cannot at present be undertaken. In the Insecta only the vagina and ejaculatory ducts with their respective accessory glands arise from the ectoderm. These structures are median and unpaired in all insects except the Ephemeridea, one of the oldest and most primitive groups. In this group, as Palmén has shown ('84), the ducts of both sexes

have independent openings. The oviducts open at the posterior edge of the seventh, the deferent ducts, which are continued into a pair of penes, at the posterior edge of the ninth segment. There is no ductus ejaculatorius proper since, according to Palmén, the chitinous cuticula covering the surface of the body does not extend in beyond the lips of the orifices.[1] In the females of *Heptagenia* the oviducts open at the bottom of an infolding of the hypodermis between the seventh and eighth segments. This infolding, the ovivalvula, accommodates the mature eggs till the time for oviposition, and may be regarded as a structure on the way to becoming a vagina. Morphologically it is simply an intersegmental depression differing from those which separate the sternites of other segments only in being somewhat exaggerated. Palmén observed that the male genital ostia are not opened till the last nymphal ecdysis.

A comparison of the nymphal Ephemerid with the Orthopteran embryo is very instructive. In *Xiphidium* and *Blatta* the female ampullæ lie at the hind end of the seventh, the male at the hind end of the ninth abdominal segment. Just as the deferent ducts of Ephemerids extend into the penes and open to the exterior, so the terminal ampullæ originally extend into a pair of appendages, albeit on the tenth segment and not opening to the exterior. If the penes of Ephemerids are really modified ambulatory appendages they would be homologous with the styli of Orthoptera. The curious persistence of these appendages in existing Orthoptera may be due to their having once functioned as penes, long after the other abdominal ambulatory appendages had disappeared. It would be necessary to suppose, if this view were adopted, that the terminations of the male ducts had moved backwards. But this whole matter is very

[1] Palmén claims ('**84**, p. 82) to have found no chitinous lining in the terminal portion of the ejaculatory ducts and oviducts of Ephemerids — an observation from which he naturally infers that these ducts are mesodermal throughout their entire length. I have found, however, that there is in the nymph of a species of *Blasturus* very common in the ponds of Worcester, Mass., a distinct chitinous lining to the ejaculatory ducts for some distance inward from the orifice of either penis. My attention was attracted to this lining during the ecdysis of the insect, when I saw the membrane withdrawn from the ducts along with the cuticle covering the external surfaces of the penes and terminal abdominal segments.

obscure, for why should the ampullæ in *Xiphidium* move from the tenth into the ninth segment? The answer to this enigma depends on further comparative embryological research. The long persisting closure of the ostia of the male ducts in Ephemerids is probably an embryonic trait. That the vagina and ejaculatory duct of higher insects may have arisen from a simple intersegmental depression like the ovivalvula receives some support from the fact that the ectodermal portions of the sexual apparatus make their appearance so late ontogenetically. To obtain in *Xiphidium* a condition essentially like that in Ephemerids it would only be necessary to have each terminal ampulla in both sexes open to the exterior.

The original termination of the sexual ducts in modified ambulatory appendages — which is so clearly seen in both sexes in embryonic Orthoptera — is very probably a primitive feature. In the Malacostraca among Crustacea and in Diplopod Myriopoda the sexual ducts terminate on more or less modified ambulatory limbs; in both sexes in the former group, only in the males in the latter. In the insect embryo the male genital appendages are larger than those of the female; hence, perhaps the larger size of the ampullæ filling their cavities. The ampullæ are probably very important structures from a phylogenetic standpoint. They may perhaps represent the nephridial Endblasen of *Peripatus* and *Annelids*, providing these latter structures are mesodermal. In Annelids the Endblasen occasionally function as temporary receptacles for the sexual products, a function which seems to have been retained in the male insect, where they become the vesiculæ seminales.

Within the group Eutracheata[1] the position of the sexual openings is subject to great variation. Thus in Diplopods and Pauropods the ducts open behind the second pair of legs, usually between the second and third segments. In Chilopoda, on the other hand, they open on the penultimate segment. In the Symphyla the unpaired genital orifice is situated on the fourth segment, which probably corresponds to the first abdominal segment in insects. Even within the division Apterygota great variation is observable. In the Collembola in

[1] Under this heading I would include the Myriopoda and Hexapoda.

both sexes the orifice lies at the posterior edge of the fifth abdominal segment. In the Thysanura the female opening usually lies on the eighth, and that of the male on the ninth abdominal segment. In female Orthoptera and Ephemeridea the sexual organs open behind the seventh, in the male behind the ninth abdominal segment, while in the Plecoptera and many other insects the female orifice is said to lie at the posterior edge of the eighth segment. Although these facts of adult anatomy point to great instability in the segmental termination of the sexual ducts, the evidence from embryology is more conclusive. The female *Xiphidium* embryo has at first two pairs of ducts, and in the male the single pair shift their position from the tenth to the ninth segment. The former fact proves conclusively that the male and female ducts are not homologous but homodynamous structures, and the latter that ducts may shift their insertions from one segment to another during ontogeny. The inference is, that sexual ducts may arise in any nephridium-bearing segment from the pair of nephridia which best subserve the sexual function and at the same time interfere least with the development and function of other organs, and that a phylogenetic shifting of the ducts has probably taken place repeatedly. The position of the genital ostia on a particular segment cannot therefore be regarded as a character of high morphological value, at least for the larger groups.

Two conflicting views have long been entertained respecting the morphological significance of the gonapophyses. Under this term, introduced by Huxley ('77), we may include the appendages of the eighth to the tenth abdominal segments in the female and such of their homologues as persist in the male. In the female, these appendages go to form the ovipositor. According to Lacaze-Duthiers ('49–'53) they are not true appendages, *i. e.* homodynamous with the legs, mouth-parts, etc., but simply modified ventral sclerites. Haase ('89), too, believes that the gonapophyses are not true appendages but "Integumentbildungen von etwas höherer Werthigkeit als die Griffel," or styloid processes which are found inserted at the bases of the legs in some Myriopods and Thysanura. A similar view ap-

pears to be held by Grassi ('89). All these authors base their conclusions solely on comparative anatomical data.

Other observers, including Weismann ('66), Huxley ('77), Uljanin, Kowalevsky ('73), Kraepelin ('72), Dewitz ('75) and Cholodkovsky ('91[a]) regard the gonapophyses as homodynamous with the true ambulatory appendages. Most of these authors adduce support for their views from the origin of the ovipositor during the larval and pupal stages. The ovipositor and sting have been traced in Orthoptera and Hymenoptera to two pairs of imaginal disks — one situated on the eighth, the other on the ninth abdominal segment. On the latter segment the pair of disks gives rise to a bifurcate or double pair of appendages. (Dewitz, Kraepelin, etc.) But the mere fact that these appendages arise from imaginal disks is not sufficient evidence of their homodynamy with ambulatory appendages, since the wings of the Metabola also arise from imaginal disks, yet cannot belong to the same category as the ambulatory appendages. The imaginal disks of the gonapophyses must be traced into the embryo and a connection clearly established between them and the embryonic appendages, before the view advocated by Huxley, Uljanin and others can be said to rest on a secure foundation. *Xiphidium* supplies this hitherto missing evidence. In this form there can be no doubt concerning the direct continuity of the embryonic appendages with the gonapophyses. One embryo which had just completed katatrepsis still showed traces of all the abdominal appendages. The pairs on the eighth, ninth and tenth segments were somewhat enlarged. In immediately succeeding stages the appendages of the second to sixth segments disappear ; the pair on the seventh disappear somewhat later. Up to the time of hatching the gonapophyses could be continuously traced, since in *Xiphidium* there is no flexure of the abdomen as in other forms to obscure the ventral view of the terminal segments. From the time of hatching Dewitz ('75) has traced the development of the ovipositor in another Locustid (*Locusta viridissima*) so that now we have the complete history of the organ.

While there can be no doubt about the appendages of the eighth and ninth segments, which go to form the two outer

sheaths of the ovipositor or sting, the development of the innermost pair of blades is by no means so satisfactory. But whether this pair is only a portion of the ninth pair of appendages, as most authors claim, or represents the tenth pair of appendages, as I maintain, the main question at issue is in no wise affected; for it still remains true that the ovipositor consists of two or three pairs of modified ambulatory limbs.

In the male *Xiphidium* embryo it was claimed that the pair of appendages on the ninth segment persists to form the definitive styli; those of the eighth and tenth segments disappearing very early. The continuity of the styli with the embryonic appendages was quite as satisfactorily observed as the continuity of the ovipositor blades. Cholodkowsky has made an exactly similar observation on *Blatta* ('91[a]). The styli are, therefore, the homologues of the second pair of gonapophyses. Haase must therefore have gone astray in seeking to homologize the styli with the styloid processes, or "Griffel," for the styli are modified ambulatory appendages. Moreover, if my interpretation is correct, he cannot have found, as he claims, the evanescent rudiments of styli in young female Blattids, since the second pair of "anal palps" are the homologues of the styli (*vide* Huxley, '77).

VII. The Subœsophageal Body in Xiphidium and Blatta.

This structure, of which I have elsewhere ('92) given a brief preliminary account, makes its appearance in the *Xiphidium* embryo, in a stage a little earlier than F. The somites in the oral and thoracic segments are then established as closed sacs. The stomodæum is still a relatively shallow depression, and the entoderm-bands starting from its inner end have made but little progress. Sagittal (Fig. 61) and frontal sections (Fig. 62), through the heads of embryos in Stage F, show several interesting details. A pair of somites (*coe*) lie in the mandibular segment, and previous to this stage there was also a pair of small somites with indistinct cavities in the tritocerebral segment (*tc*). The planes of section in the two figures are such that the deutocerebral somites are not shown. A mass

of cells, the subœsophageal body, colored pink in the figure, extends between the œsophagus and the mandibular somites. The origin of this mass is obscure. It may arise from the ectoderm of the œsophagus, to the inner end of which it is attached (Fig. 61), or it may come from the entoderm (*en*). I believe, however, that it arises from neither of these sources, but from the mesoderm, which in a preceding stage formed the abortive somites of the tritocerebral segment. In frontal section (Fig. 62) the mass of cells is Λ-shaped, with the juncture of its two arms attached to the lower surface of the œsophagus. The distal ends of the arms are applied to the anterior walls of the mandibular somites. The separate cells are often sharply wedge-shaped and appear to be separated by clear spaces. They grow somewhat, lose their triangular outline and become more rounded. At the same time they tend to fuse in curved strings, with their broad edges applied to one another. This condition is seen in Fig. 63, which is taken from a section through the organ of an embryo in Stage G. The cytoplasm is now very granular, and has a distinctly yellow tint even in unstained sections; like the neuroblasts and germ-cells it absorbs picric acid with avidity. Vacuoles have begun to make their appearance, and the walls between adjacent cells are disappearing. The volume of the nuclei remains constant, but the cytoplasm enlarges up to the time of hatching. Fig. 64 is a part of a section through the subœsophageal body of a 7 mm. larva of *Xiphidium fasciatum*. The condition of the organ is essentially the same as at the time of hatching. The increase in volume of the cytoplasm is clearly shown. Instead of being granular, as in the younger stages, the protoplasm is now so filled with small vacuoles that it is reduced to a coarse reticulum.

In the subœsophageal body of a larva 9 mm. long, signs of degeneration have begun to appear. The small vacuoles fuse in the centres of the cells, leaving only the cell-walls as ragged envelopes. The nuclei become somewhat polygonal in outline. At this time the organ is found attached to the anterior ends of the salivary glands and to the large trunks which run forward into the head from the first thoracic tracheæ.

A section from a very young nymph (10 mm. long), is shown in Fig. 65. The cytoplasm of the fused cells is reduced to a ragged mass in which the irregular nuclei are suspended. Their chromatin is aggregated in rounded masses — a sign of advanced degeneration. In this stage the organ is much shrunken in size so that one is led to conclude that part of it has already been absorbed. In a little later stage the last traces of the organ have disappeared.

A subœsophageal body essentially like the one here described occurs also in *Blatta*. It, too, has the characteristic yellow tint. In his study of the development of *Blatta* Cholodkowsky appears to have seen this peculiar structure, though he regarded it as a portion of the fat-body. At page 52 ('91[a]), he says: "Die Entodermlamelle umwächst den Nahrungsdotter dorsalwärts und von allen Seiten; der Vorder- und Hinterdarm liegen nun ausserhalb des Nahrungsdotters und werden vom homogenen Dotter umspült, in *welchem (besonders neben dem Œsophagus) kleine blasse Zellen liegen, die sich in Fettkörper zu verwandeln scheinen.*"[1] The organ is shown in Cholodkowsky's Fig. 68, Pl. VI. In other writers on insect embryology I find no mention of this interesting structure.

In the Rhynchota, to judge from a few observations on the embryos of *Zaitha flumínea*, the subœsophageal body occurs in a slightly modified form. Here it consists of a number of loose spherical cells lying on either side and a little below the œsophagus. The nuclei are large and spherical and the compact and finely granular cytoplasm has a distinct yellow cast. Though these cells vary in size (11–15μ) they are always larger than the cells of the surrounding tissues (6.3μ). Beyond this stage I could not trace the organ in *Zaitha* on account of lack of material.

The subœsophageal body may always be readily distinguished from the fat-body of the oral and more posterior segments by the peculiar structure and arrangement of its cells and by its yellow tint. I therefore regard it as an organ *sui generis*. It belongs to the category of embryonic or early larval organs,

[1] The italics are mine.

and this alone would suffice to distinguish it from the fat-body which persists throughout life.

It is perhaps premature to advance any hypothesis as to the function and morphological significance of the subœsophageal body, but I may call attention to its possible homology with an organ in the Crustacea. The researches of Viallanes and St. Remy go to show that the tritocerebral segment of insects is homologous with the second antennary segment of Crustacea. In the latter group of Arthropods this segment is provided with the green-gland, a structure which develops from the mesoderm and is generally regarded as a modified nephridium. The subœsophageal body, providing it arises from the mesoderm of the tritocerebral segment, may be all that remains of this same pair of nephridia in the cephalic region of insects.

VIII. Technique.

Xiphidium eggs, like those of other Orthoptera are not easily sectioned in the younger stages, because their yolk bodies are rendered so brittle by the hardening fluids and are cemented together with so little protoplasm that they disintegrate during the process of cutting. After the appearance of the appendages the embryo may be readily dissected away from the yolk either in the fresh or hardened egg and mounted or sectioned by itself. In the study of the envelopes, where it is necessary to section the whole egg, the following method gives fairly good results :—

The eggs are taken from the galls and killed by being placed for about a minute in water heated to 80° C.[1] They are then transferred for preservation to 70 per cent alcohol in which they should remain for several weeks, if not months, in order to allow the yolk to harden and to shrink away from the chorion. The neglect of this simple precaution has led many to exaggerate the difficulty of studying insect eggs or to abandon them altogether. After remaining in the alcohol for some time, the chorion may be removed by tearing it

[1] Alcoholic picrosulphuric acid also proved to be an excellent killing reagent.

open at the broad pole and gently pushing against the narrow pole of the yolk with one needle, while holding on with the other to the chorion at the same pole. In the earliest and latest stages the chitinous blastodermic membrane comes off with the chorion, in other stages it adheres firmly to the yolk and prevents satisfactory staining. If aqueous stains like Orth's lithium carmine or Grenacher's alum carmine are used, the eggs should be left in them but a short time and carefully watched as the yolk-bodies have a peculiar tendency to absorb water till they lose the polygonal shapes they acquired by mutual pressure, finally swell and fall asunder. This is especially liable to occur in the younger stages when the blastodermic membrane is removed. I have as yet found no other insect egg with yolk capable of imbibing so much water. In Grenacher's borax carmine there is no swelling, a reason which has induced me to use this stain in preference to the aqueous solutions; though the two stains mentioned give excellent results if used with due precautions. After dehydrating and clearing with cedar oil, the eggs are kept from two to three hours in melted paraffine (55° C.). Older embryos in which most of the yolk has been metabolized need not remain in paraffine more than an hour.

Embryos isolated from the yolk in the anatreptic stages, as well as later embryos used in sectioning, were stained in Czokor's alum cochineal. The bluish color of this stain is preferable to the borax carmine in serial sections, as it is less wearisome to the eye.

In the study of the entire embryo three different methods may be followed with advantage.

Method I. — The isolated embryo is stained with borax carmine, all excess of the stain is removed by prolonged immersion in acid alcohol, and the preparation mounted in clove oil or balsam. In such preparations many of the details of internal structure, such as the arrangement of the cœlomic sacs, may be very clearly distinguished. This method was very extensively used by Graber; in fact it seems to have been the only method which he employed for surface study. In this respect it is decidedly inferior to

METHOD II. — The hardened eggs or embryos, freed from their envelopes, are transferred from seventy per cent alcohol to Delafield's or Ehrlich's hæmatoxylin, in which they are left not longer than thirty or forty seconds. Then they are suddenly returned to seventy per cent alcohol, and a drop of twenty per cent HCl is allowed to fall through the alcohol onto the embryos, which almost instantly change color. As soon as they pass from a red to a salmon tint the fluid must be hastily removed and replaced by fresh seventy per cent alcohol, to which a trace of ammonia has been added. The nuclei gradually turn blue and throw the embryo out in bold contrast to the pale yellow yolk. In older isolated embryos, the stain faintly tinges the surface protoplasm, accentuates the shadows, and leaves all the sharp depressions unstained. When embryos thus treated are mounted in glycerin or balsam and examined with widely opened diaphragm and Abbé condensor under a moderately low power (about sixty diameters), the surface relief is exquisitely sharp and clear. The exact delimitation of the appendages, both permanent and evanescent, the tracheal orifices, œnocytic invaginations, segments of the brain and nerve-cord, etc., may be traced with great precision, as the figures on Plate I will testify.

The method here given with several modifications of my own, was taught me by Dr. Wm. Patten, who has used it with great success in his studies of Arthropod development, more especially in his work on the brain and eye of *Acilius*. A very similar method seems to have been used by other investigators (*vide* Foster and Balfour, Elements of Embryology, 1883). Unfortunately, surface preparations with hæmatoxylin are not permanent, probably on account of the acid used to extract the stain. The color gradually fades, often disappearing completely in the course of a few weeks. I therefore prefer Czokor's alum cochineal, washing in water instead of acidulated alcohol. These preparations are nearly or quite as clear as the hæmatoxylin preparations and keep indefinitely.

METHOD III. — This is really only a compromise between Methods I and II. Embryos in the katatreptic stages are allowed to remain in Czokor's alum cochineal till the stain has

penetrated as far as but not into the yolk. They are then washed in water, dehydrated and mounted in balsam. The sexual ducts together with their ampullæ may be distinctly traced on the yellow background of the yolk and structures which lie just beneath the integument, like the œnocyte clusters and the nerve-cord, may be more readily studied than in specimens prepared by Methods I and II. The figures on Plate V and Fig. 10, Plate I were drawn from such partially stained embryos.

The methods here described give good results, not only with *Xiphidium* and *Blatta*, but also with all the other insects and crustaceans which I have examined.

The outlines of the figures in the plates were drawn with an Abbé camera.

Clark University,
Worcester, Mass., May 10th, 1892.

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EXPLANATION OF PLATE I.

(*Xiphidium ensiferum*, *Scud.*)

FIG. 1 (*A*). Surface view of embryo during gastrulation. *p.o.*, indusium; *pc.l*, procephalic lobe; *bl.*, blastopore; *a.*, anal bifurcation of the blastopore; *ams.*, amnioserosal fold.

FIG. 2 (*B*). Surface view of embryo with amnioserosal fold closed over trunk-region. *pcl.n.*, neuroblast-centres on the procephalic lobes. *o.*, anterior widening of the blastopore. Remaining letters as in Fig. 1.

FIG. 3 (*C*). Surface view of embryo with the amnioserosal fold encroaching on the indusial thickening; *po.am.*, amnioserosal fold of the indusium; *z.*, pedicel temporarily uniting the indusium with the head; *at.*, antenna; *md.s.*, mandibular segment; *mx.s.*,[1] first maxillary segment; *mx.s.*,[2] second maxillary segment; *p.s.*,[1]–*p.s.*,[3] first to third thoracic segments; *a.s.*,[1] first abdominal segment. Remaining letters as in Fig. 1.

FIG. 4 (*D*). Surface view of embryo just after the separation of the indusium from the head. Letters as in Figs. 3 and 1.

FIG. 5 (*E*). Indusium spreading over the yolk. View of embryo nearly completely submerged in the yolk, on its way to the dorsal surface. *tc.s.*, tritocerebral segment. Other letters as in preceding figures.

FIG. 6 (*F*). Surface view of elongate embryo on dorsal surface of yolk. *lb.*, labrum; *md.*, mandible; *mx.*,[1] first maxilla; *mx.*,[2] second maxilla; *p.*[1]—*p.*,[3] the three thoracic appendages (legs); *coe.*, cœlomic sac of first abdominal segment showing through the body wall; *pl.* (*ap.*[1]), pleuropodium (appendage of the first abdominal segment); *v.*, yolk; *envl.*, cellular envelopes torn away from the ventral face of the embryo; *cc.* (*ap.*[11]), cerci (appendages of the eleventh abdominal segment).

FIG. 7 (*G*). Surface view of shortened embryo on dorsal yolk. *pc.*,[2] second protocerebral lobe; *pc.*,[3] third protocerebral lobe; *dc.*, deutocerebrum; *tc.*, tritocerebrum; *e.*, eye; *x.*, metastigmatic, or œnocytic invagination; *ap.*,[4] fourth abdominal appendage. Remaining letters as in Fig. 6 (*F*).

FIG. 8 (*H*). Surface view of embryo turning the lower pole of the egg. *sr.*, inner indusium functioning as the serosa; *am.*, amnion reflected back over the yolk and continuous with the membrane *sr.*; *at.*, antenna; *pl.*, right pleuropodium; *igl.*, intraganglionic thickening.

FIG. 9 (*J*). Surface view of embryo just after returning to the ventral face of the egg. *e.*, eye; other letters as in Fig. 8 (*H*).

FIG. 10 (*K*). Surface view of advanced ♀ embryo just before the secretion of the larval cuticle. *d.o.*, "dorsal organ"; *f.d.*, oviduct; *ta.*, terminal ampulla of oviduct; *op.*[1] (*op.*[8]), *op.*[2] (*ap.*[9]), first and second pairs of gonapophyses (appendages of the 8th and 9th abdominal segments). *cc.* (*ap.*[11]), cercus.

EXPLANATION OF PLATE II.

(Figs. 11 and 12, *Stagmomantis carolina;* Figs. 13 and 14, *Gryllus luctuosus;* Figs. 15–20, *Xiphidium ensiferum.*)

FIG. 11. Surface view of gastrula of *Stagmomantis.* × 150. *p.o.*, rudiment of indusium?; *bl.*, blastopore; *ams.*, amnioserosal fold extending just over the edge of the oval germ-band.

FIG. 12. Outline of egg of *Stagmomantis* showing the position and relative size of the germ-band during gastrulation.

FIG. 13. Surface view of gastrula of *Gryllus.* × 150. *ams.*, amnioserosal fold extending just over the edge of the germ-band; *bl.*, deeper posterior end of blastopore.

FIG. 14. Outline of egg of *Gryllus* showing the relative size and position of the germ-band during gastrulation.

FIG. 15. Surface view of a *Xiphidium* embryo during the closure of the amnioserosal fold over the mouth. Eight segments in the trunk. *p.o.*, indusium; *y.*, pale area between the indusium and the head; *pc.l.*, procephalic lobe; *ams.*, edge of amnioserosal fold. *o.*, stomodæum; *md.s.*, mandibular segment; *mx.s.*[1], first maxillary segment; *n.g.*, neural groove; *s.*, serosal nuclei; *a.s.*,[1] first abdominal segment.

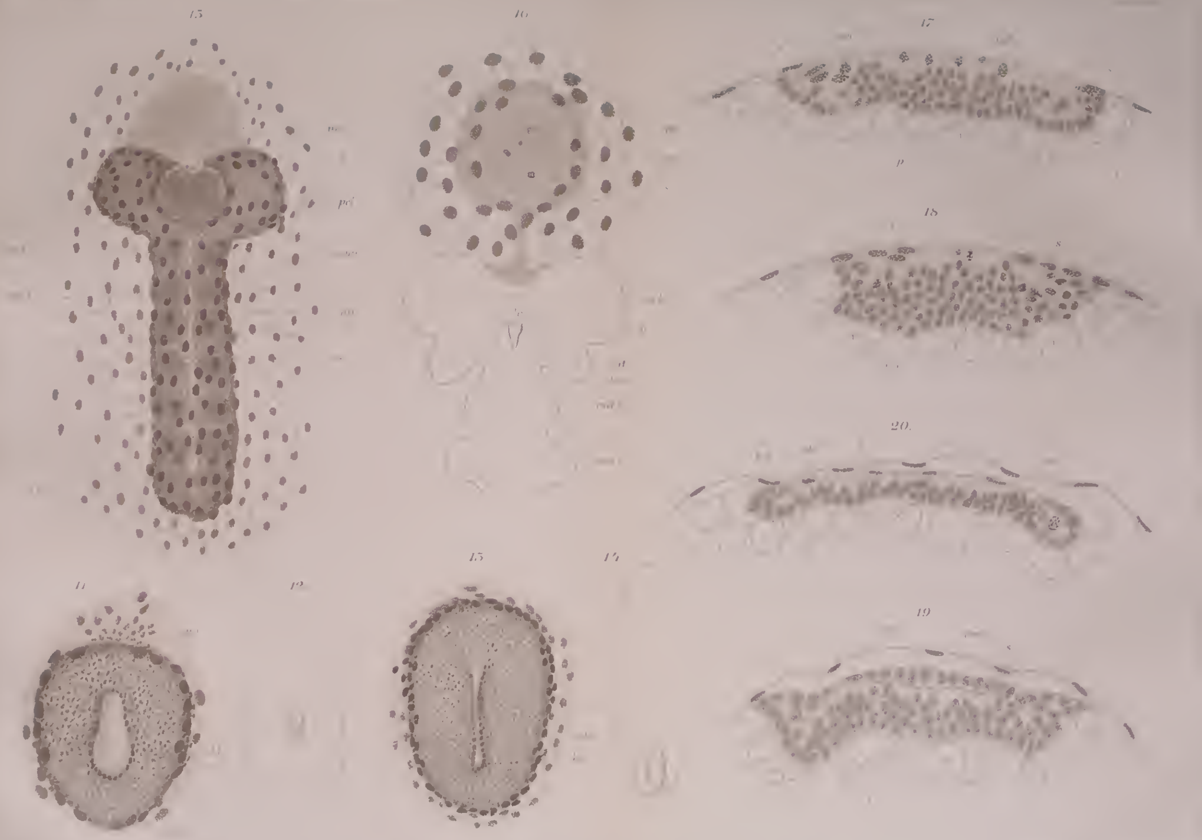
FIG. 16. Surface view of head during the separation of the indusium. Stage of Fig. 3 (*C*), Plate I more highly magnified. *p.o.*, indusium; *nn.*, shrunken nuclei of the indusium; *s.*, serosal nucleus; *z.*, pedicel connecting the indusium with the head; *pc.l.*, procephalic lobe; *lb.*, labrum; *o.*, stomodæum; *at.*, antenna; *tc.s.*, tritocerebral segment; *md.s.*, mandibular segment; *mx.s.*,[1] first maxillary segment.

FIG. 17. Median transverse section through the indusium while still a simple thickening of the blastoderm (serosa). × 230. *s.*, serosa; *p.o.*, portion of indusium with normal nuclei; *nn.*,[1] shrunken nuclei; *nn.*,[2] less shrunken nuclei; *d.*, thickened periphery of the organ; *v.*, yolk.

FIG. 18. Median transverse section of the indusium while the amnioserosal fold is closing over the disk. × 230. Letters as in Fig. 17.

FIG. 19. Median transverse section through the indusium after the complete closure of the amnioserosal folds. × 230. *am.*,[1] outer indusial layer; *p.o.*, inner indusial layer; *nn.*,[1] shrunken nuclei.

FIG. 20. Median transverse section through the indusium just after it has begun to spread. × 230. Letters as in the preceding figures.



EXPLANATION OF PLATE III.

(*Xiphidium ensiferum.*)

FIG. 21. Median longitudinal section through the head of an embryo over which the envelopes have just closed. × 230. *o.*, stomodæum; *am.*, amnion; *s.*, serosa; *p.o.*, indusium; *z.*, pedicel uniting the indusium to the head of the embryo; *ec.*, ectoderm; *ms.*, mesoderm; *vph.*, vitellophag; *v.*, yolk.

FIG. 22. Median tranverse section of the indusium when it has reached half way round the egg. × 230. *p.o.*, inner indusial layer; *am.*,[1] outer indusial layer; *s.*, serosa.

FIG. 23. Three normal cells from the indusium. × 700.

FIG. 24. Three cells with shrivelled nuclei from the indusium. × 700.

FIG. 25. Transverse section through basal abdominal region of an embryo passing to the dorsal surface of the yolk. × 230. *nb.*, neuroblasts; *db.*, dermatoblasts; *am.*, amnion; *ms.*, mesoderm; *ec.*, ectoderm.

FIG. 26. Transverse section through first maxillary segment of an embryo passing to the dorsal surface of the yolk. × 230. *ng.*, neural groove; *en.*, entoderm. Other letters as in Fig. 25.

FIG. 27. Transverse section through the second maxillary segment of an embryo in the stage of Fig. 6, (*F*) Plate I. × 230. *mx.*,[2] second maxilla (trilobed); *mnb.*, median-cord neuroblast; *ps.*, Punktsubstanz; *vph.*, vitellophag. Other letters as in Figs. 25 and 26.

FIG. 28. Transverse section through the mesothoracic ganglion of an embryo in a stage somewhat younger than G (Fig. 7, Pl. I). × 230. *g.*,[1] younger offspring of the neuroblasts; *g.*,[2] older offspring of the neuroblasts (ganglion cells); *ecd.*, ectoderm; *mc.*, median cord ("mittelstrang"). Remaining letters as in preceding figures.

FIG. 29. Sagittal section through nerve-cord a little to one side of the median line. Embryo in Stage G (Fig. 7, Pl. I). × 175. *md.g.*, mandibular ganglion; *mx.g.*,[1] first maxillary ganglion; *mx.g.*,[2] second maxillary ganglion; *ig.*,[1] *ig.*,[2] first and second interganglionic depressions; *p.g.*,[1] first thoracic ganglion; *p.g.*,[2] second thoracic ganglion; *mnb.*, median cord neuroblasts; *mg.*, their progeny; *inl.*, inner neurilemma.

FIG. 30. Frontal section through the base of the abdomen of an embryo somewhat older than Fig. 6, (F), Pl. I. × 175. *nb.*, neuroblasts; *mnb.*, median cord neuroblasts in the intersegmental regions; *p.*,[3] metathoracic leg; *pl.*, pleuropodium; *ap.*,[2]–*ap.*,[5] second to fifth abdominal appendages grazed by the knife.

FIG. 31. Transverse section through the mesothoracic ganglion of an embryo in a stage between Fig. 9 (*J*) and 10 (*K*) Pl. I. × 230. *hy.*, hypodermis (product of dermatoblasts); *nb.*, neuroblasts; *g.*[n], latest progeny of the neuroblasts; *g.*, ganglion-cells; *inl.*, inner neurilemma; *enl.*, outer neurilemma; *ps.*, Punktsubstanz.

EXPLANATION OF PLATE IV.

(*Xiphidium ensiferum.*)

FIG. 32. Transverse section through the head of an embryo in the stage of Fig. 5 (E) Pl. I. × 230. *lb.*, labrum; *pc.*[1] (*o.g.*), *pc.*,[2] *pc.*,[3] first, second and third protocerebal lobes; *o.p.*, optic plate; *en. ms.*, mesentoderm.

FIG. 33. Next following section to that represented in Fig. 32. ×230. *n.b.*, neuroblast; *am.*, amnion. Remaining letters same as in Fig. 32.

FIG. 34. Next following section to that represented in Fig. 33. × 230. *st.*, stomodæum. Remaining figures the same as in Figs. 32 and 33.

FIG. 35. Transverse section through the labrum, in a stage intermediate between E and F (Figs. 5 and 6, Pl. I.) × 230. *pc.*,[3] third protocerebal lobe; *lb.*, labrum; *en. ms.*, mesentoderm.

FIG. 36. Transverse section through labrum and brain of an embryo in Stage F (Fig. 6, Pl. I.) × 230. *lb.*, labrum; *pc.*,[1] *pc.*,[2] *pc.*,[3] first, second and third protocerebral lobes; *op.*, optic plate; *nb.*, neuroblast; *db.*, dermatoblasts; *coe.*, head-cœlom; *ms.*, mesoderm cells; *igl.*, intraganglionic thickening; *st.*, stomodæum; *am.*, amnion.

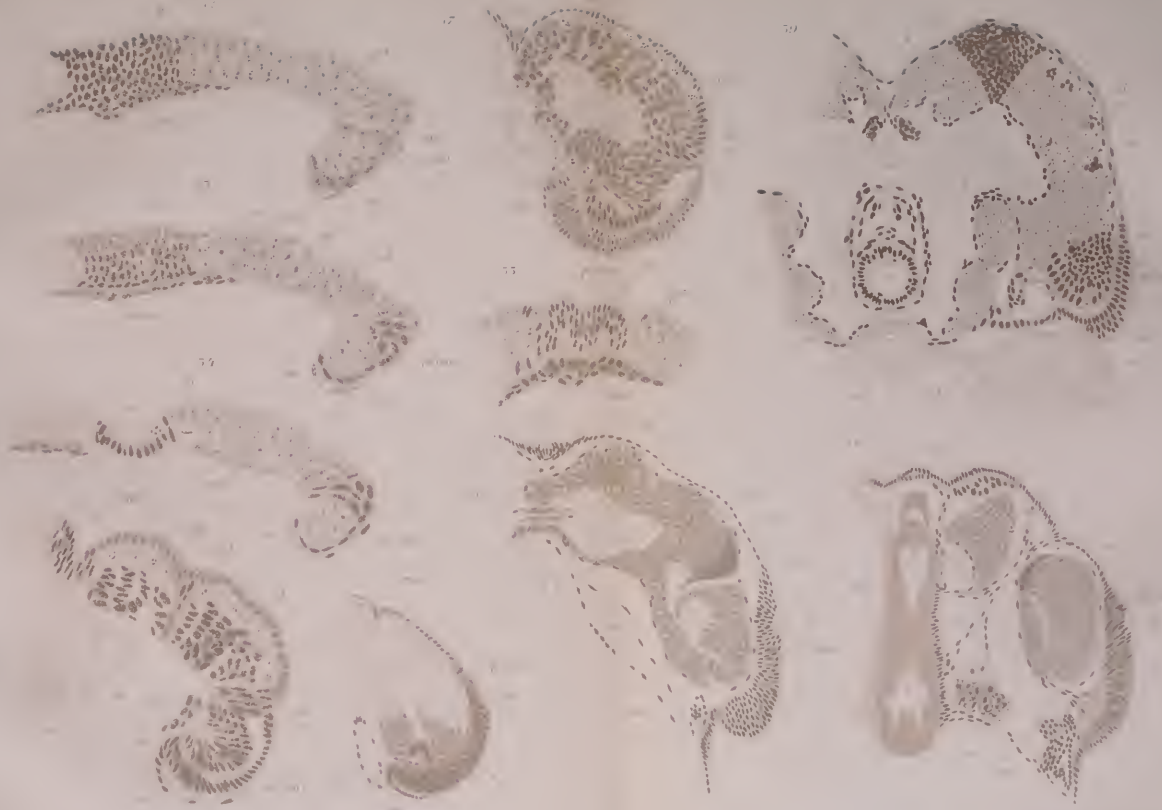
FIG. 37. Transverse section through prælabral region of an embryo in stage somewhat later than F (Fig. 6, Pl. I). × 145. *mc.*, median cord; *pc.*[1] (*o.g.*), first protocerebal lobe (optic ganglion); *w.*, orifice of involution of the intraganglionic thickening. Remaining letters as in Fig. 36.

FIG. 38. Transverse section through optic ganglion and optic plate of an embryo in Stage G (Fig. 7, Pl. I). × 145. *th.*, clear thickening in the optic plate; *e.*, eye; *on.*, optic nerve; *pc.*[1] (*o.g.*), optic ganglion; *am.*, amnion.

FIG. 39. Frontal section through the brain of an embryo somewhat older than I (Fig. 9, Pl. I). × 175. *p.*, problematical brain segment; *pc.*[1] (*o.g.*), optic ganglion; *pc.*,[2] *pc.*,[3] second and third protocerebral lobes; *dc.*, deutocerebrum; *tc.*, tritocerebrum; *e.*, eye; *md.*, mandible; *md.g.*, mandibular ganglion; *r.g.*, recurrent ganglion; *st.*, stomodæum.

FIG. 40. Transverse section of brain through the supracœsophageal commissure of an embryo in Stage K (Fig. 10, Pl. I). × 175, *nb.*, neuroblasts; *igl.*, interganglionic thickening; *th.*, clear thickening in the optic plate; *coe.*, head-cœlom; *ps.*, Punktsubstanz; *on.*, optic nerve. Remaining letters as in Fig. 39.

FIG. 41. Transverse section from same series as that represented in Fig. 40, but passing through the frontal ganglion. × 175. *dc.*, deutocerebrum; *f.g.*, frontal ganglion; *sb.*, subœsophageal body. Remaining letters as in Figs. 39 and 40.



EXPLANATION OF PLATE V.

(*Xiphidium ensiferum*, Figs. 42–46, 48–50. *X. fasciatum*, Figs. 47 and 51.)

FIG. 42. Tip of abdomen in surface view from a ♂ embryo which has just passed the lower pole. (Stage J, Fig. 9, Pl. I.) t^5–$t.^8$ fifth to eighth abdominal stigmata; *ts.*, testis; *m.d.*, vas deferens; *ta.m.*, terminal ampulla; *ap.*,[8] appendages of eighth abdominal segment; *st.* (*ap.*[9]), stylets (appendages of ninth abd. seg.; *ap.*,[10] appendages of tenth abdominal segment, in the cavities of which the ampullæ lie in this stage; *cc.* (*ap.*,[11]) cerci.; *prd.*, proctodæum; *an.*, anus.

FIG. 43. Tip of abdomen in surface view from a ♂ embryo somewhat older than the one shown in Fig. 42. Letters same as in Fig. 42.

FIG. 44. Tip of abdomen in surface view from a ♂ embryo somewhat older than the one shown in Fig. 43. *v.*, yolk; *ag.*, last abdominal ganglion. Remaining letters as in Fig. 42.

FIG. 45. Vasa deferentia (*m.d.*), with their terminal ampullæ (*ta.m.*), from an embryo just before the development of the larval cuticle.

FIG. 46. Tip of abdomen of a ♂ embryo ready to hatch. Letters same as in Figs. 42–44.

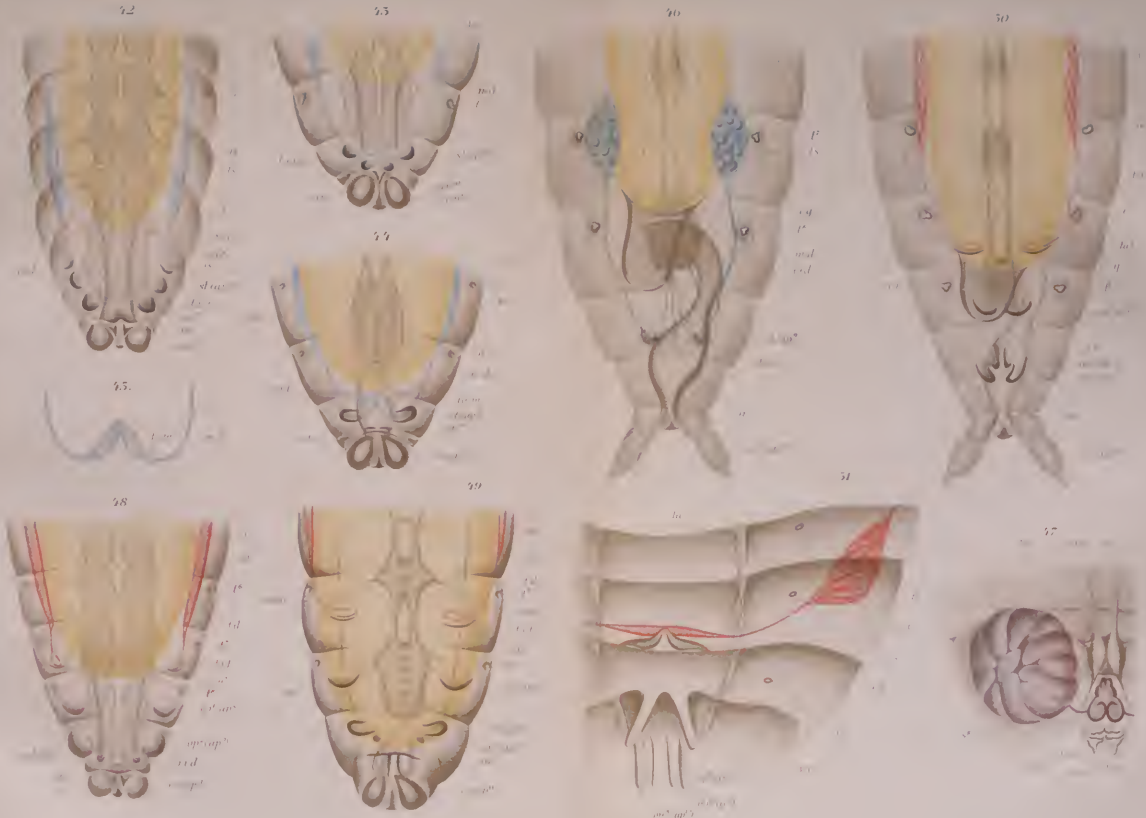
FIG. 47. Tip of abdomen opened and seen from within from a ♂ larva 1 cm. long. *m.o.*, sexual orifice, *c.n.*, connectives; remaining letters as in Figs. 42–44.

FIG. 48. Tip of abdomen of ♀ embryo in a stage corresponding to that represented in Fig. 42. *ov.*, ovary; *f.d.*, oviduct; *ta.f.*, terminal ampulla; *m.d.*, vas deferens and terminal ampulla of the male type, still persisting; $t.^6$–$t.^8$, sixth to eighth abdominal stigmata; *ap.*,[7] persisting appendage of the seventh abdominal segment; *op.*[1] (*ap.*[8]), *op.*[2] (*ap.*[9]), *op.*[3] (*ap.*[10]), three pairs of abdominal appendages which become the gonapophyses (ovipositor); *an.*, anus; *cc.* (*ap.*[11]), cerci; *v.*, yolk.

FIG. 49. Tip of abdomen of ♀ embryo seen in full surface view in Fig. 10. (*K*), Pl. I. *mnb.*, location of median cord neuroblast; *cm.*, posterior commissure; *cn.*, connective; *ag.*, last abdominal ganglion. Other letters as in Fig. 48.

FIG. 50. Tip of abdomen of ♀ embryo ready to hatch. Letters same as in Figs. 48 and 49.

FIG. 51. Tip of abdomen of ♀ larva 1 cm. long, opened and seen from within; all the parts being dissected away except the reproductive organs. *vg.*, vagina; *op.*[1] (*ap.*[8]), *op.*[2] (*ap.*[9]), *op.*[3] (*ap.*[10]), three pairs of gonapophyses. Other letters as in Fig. 48.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EXPLANATION OF PLATE VI.

(*Xiphidium ensiferum*, Figs. 52–63; *X. fasciatum*, Figs. 64 and 65).

FIG. 52. Frontal section through first to fourth abdominal segments, showing segmental arrangement of the gonads. Embryo in Stage F (Fig. 6, Pl. I). × 230. *ml.*, longitudinal ventral muscle; *gd.*^{1}*gd.*^{2}*gd.*,^{3} gonads of first, second and third abdominal segments; *coc.*, cœlomic cavity; *ecd.*, ectoderm; *ep.*, epithelium.

FIG. 53. Transverse section through the third abdominal segment of an embryo in Stage F, (Fig. 6, Pl. I). × 230. *nc.*, nerve cord; *am.*, amnion, *sms.*, somatic wall of somite; *spms.*, splanchnic wall of somite; *v.*, yolk; *en.*, entoderm. Remaining letters as in Fig. 52.

FIG. 54. Frontal section through the fourth abdominal segment of an embryo in Stage F (Fig. 6, Pl. I). × 230. The diverticula point towards the head. *v.*, yolk; *gd.*,^{4} gonad of fourth abdominal segment; *coe.*, cœlomic cavity; *ep.*, epithelium.

FIG. 55. Section through a somite from the third thoracic segment showing a single enlarged germ-cell protruding into the cœlomic cavity. × 230.

FIG. 56. Sagittal section through the end of the abdomen of an embryo in Stage G (Fig. 7, Pl. I). *tb.*, neuroteloblast?; *nb.*, neuroblasts; *coe.*^{7}–*coe.*,^{10} cœlomic cavities of the seventh to tenth abdominal segments; *m.d.*, diverticulum of the tenth abdominal somite which becomes the vas deferens and its terminal ampulla; *gd.*,^{10} gonad in tenth abdominal segment (abnormal and atavistic); *n.c.*, nerve cord in unflexed portion of abdomen.

FIG. 57. Transverse section through ninth abdominal segment of embryo represented in Fig. 42, Pl. V, cutting the tenth pair of appendages. × 175. *msc.*, muscular tissue; *prd.*, proctodæum; *n.c.*, nerve cord; *h.*, heart; *ecd.*, ectoderm; *m.d.*, vas deferens; *ta.m.*, terminal ampulla; *ap.*,^{10} appendage of the tenth abdominal segment.

FIG. 58. Transverse section of the abdomen of an embryo in the stage represented in Fig. 48, Pl. V. The greater portion of the section passes through the ninth, its anterior portion through the tenth abdominal segment. × 175. *coe.*,^{10} cœlom of tenth abdominal segment; *bl.*, blood corpuscle; *ta.m.*, terminal ampulla of vas deferens; *m.d.*, vas deferens disintegrating; *op.*^{3} (*ap.*^{10}), third pair of gonapophyses; *n.c.*, nerve cord; *prd.*, proctodæum; *ec.*, ectoderm; *h.*, heart.

FIG. 59. Transverse section through the seventh abdominal segment, taken from the same embryo as the section in Fig. 58. × 175. *v.*, yolk; *en.*, entoderm; *bl.*, blood-corpuscle dividing; *h.*, heart; *coe.*,^{7} cœlom of the seventh abdominal segment; *f.d.*, oviduct; *ta.*, terminal ampulla; *oe.*, oenocytes; *ec.*, ectoderm; *nc.*, nerve-cord; *ap.*,^{7} appendage of seventh abdominal segment.

FIG. 60. Transverse section through the seventh abdominal segment of an embryo somewhat older than that in Fig. 48, Pl. V. × 175. *ov.*, ovary; *mc.*, median cord; *ad.*, fat-body; *spms.*, splanchnic mesoderm. Other letters as in Fig. 59.

FIG. 61. Sagittal section through the head of an embryo in Stage F (Fig. 6, Pl. I). × 175. *f.g.*, frontal ganglion; *r.g.*,^{1} first recurrent ganglion; *r.g.*,^{2} second recurrent ganglion; *v.*, yolk; *pc.*,^{3} third protocerebral lobe; *ms.*, mesoderm; *lb.*,

labrum; *st.*, stomodæum; *tc.*, tritocerebrum; *md.g.*, mandibular ganglion; *en.*, entoderm; *s.b.*, subœsophageal body; *coe.*,[3] cœlom of mandibular segment.

Fig. 62. Frontal section through the head of an embryo in Stage F (Fig. 6, Pl. I). × 175. *pc.*[1] (*o.g*), optic ganglion; *md.*, mandible. Remaining letters as in Fig. 61.

Fig. 63. Transverse section through the subœsophageal body from an embryo in Stage G (Fig. 7, Pl. I.). × 500.

Fig. 64. Section through subœsophageal body of larva 7 mm. long. × 500.

Fig. 65. Section through the subœsophageal body of a nymph 1 cm. long. × 500.