PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Studies on the Embryology of the African Migratory Locust, Locusta migratoria migratorioides R. and F. I. The Early Development, with a New Theory of Multi-Phased Gastrulation among Insects

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X—Studies on the Embryology of the African Migratory Locust, *Locusta migratoria migratorioides* R. and F.

I—The Early Development, with a New Theory of Multi-phased Gastrulation Among Insects

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[Plates 33–35]

Contents

I—Introduction

Notwithstanding the large amount of work done on insect embryology, certain outstanding problems are still productive of much divergence of opinion. The process of gastrulation, the origin of the mid-gut epithelium, and, in short, the general problem as to how far the germ-layer theory is applicable to insects, are matters of dispute to-day. Further, the claim of Wiesmann (1926) to have found the labral

and the preantennary coelom sacs in *Carausius* (*Dixippus*) *morosus* (Orthoptera, Phasmidae) has lent a new interest to the problem of head segmentation among Arthropods in general and insects in particular.

The Orthoptera, being the most generalized group among the Pterygota, are specially suited for the investigation of these problems. The family Acrididae was chosen because its embryology has been but little worked out and also because of the ease with which the material for work was available. Among older literature only Packard's (1878–83) and Graber's (1888–91) works deal with members of this family, viz., with *Melanoplus spretus* and *Stenobothrus variabilis* respectively. During recent years, however, owing to the interest created by the locust problem, a number of papers has appeared which deal with the various aspects of the embryology of this family. These papers are by McNabb (1928), Slifer (1932–4), Nelsen (1931–4), and Else (1934). In the present series of papers it is proposed to give as complete an account as possible of the embryology of the African Migratory Locust, *Locusta migratoria migratorioides* R. and F., which is referred to, for brevity, as *Locusta migratoria*.

II—Material and Methods

The eggs used in this work were laid by *Locusta migratoria* L., sub-species *migratorioides* (Reiche and Fairmaire), which is kept breeding from generation to generation in the Entomological Field Station at Cambridge. The original stock consisted of eggs laid by this locust in the *phasis gregaria* near Khartoum. The supply was sent by Mr. A. H. Wood of the Gezira Agricultural Research Service to Dr. A. D. Imms who received them in June, 1933. They were transmitted by air mail and arrived in good condition. For the purpose of the present study, only eggs laid by locusts in the *phasis gregaria* were used. In order to obtain accurately timed stages, mating couples were transferred from breeding cages to an incubator maintained at a constant temperature of $33 \pm 0{\cdot}5°$ C and the eggs were kept under these conditions throughout the incubation period. The age of the eggs described in this paper refers to the above temperature. The sand containing the developing eggs was always kept moist by adding water to it from time to time.

The dissecting out of the early embryos from the egg was done in a mixture of equal parts of Bouin's fluid and Ringer's solution and fixation was done in Bouin's fluid. For staining embryos in whole eggs thionin gave good results while borax carmine was useless. A variety of fixatives was tried for the eggs. Of these, alcoholic Bouin for early stages and this and Carnoy's acetic alcohol (Formula No. 2) for late stages gave the best results. In order to ensure proper fixation, it was found, necessary to pierce the eggs in several places with a fine needle after placing them in the fixative.

The difficulty of sectioning the yolky locust eggs is accentuated by the presence of a thick and almost impermeable vitelline membrane. Several different methods of sectioning were tried, including the use of soft wax of M.P. 42° C before passing into

harder wax ; double embedding (collodion and wax) ; clearing in aniline oil or in methyl salicylate after 90% alcohol ; clearing in oil of turpentine after 95% alcohol ; and finally, HEIDER's method (1889) of painting with collodion. Of these the last alone gave reasonably satisfactory results, but was very slow. The process finally employed, however, was a modification of PETRUNKEWITSCH's (1933) cupric-phenol fixing method introduced by SLIFER and KING (1933) and improved by ROONWAL (1935). Sections were cut from 5 to 12 μ thick. To prevent thick sections from falling off the slide, the latter, with the sections *in situ*, was dipped into HESSE's (1901) photoxylin solution ($\frac{1}{4}$ to $\frac{1}{2}$% photoxylin in absolute alcohol and ether) before passing into 90% alcohol while descending from absolute alcohol. For staining sections, the best results were obtained with Heidenhain's iron haematoxylin and orange G.

III—EMBRYONIC DEVELOPMENT

1—*Time-table of Early Development*

The total duration of incubation at 33° C from the time of egg-laying up to the emergence of the nymph is about 12$\frac{1}{2}$–13 days. Blastokinesis occurs at the 5$\frac{1}{2}$–6 days' stage. In Table I is given a detailed time-table of the early development :—

TABLE I—TABLE OF EARLY DEVELOPMENT

Age of egg (in hours after egg-laying)	State of development
0–5$\frac{1}{2}$	Maturation and fertilization probably occur within 1–2 hours after egg-laying. First and second cleavage divisions.
5$\frac{1}{2}$	Stage with 4 cells.*
7	Stage with 6 cells.*
8	Stage with 16–18 cells.*
10	Stage with about 41–45 cells.*
13	The cells are nearing the egg-periphery and have reached half-way up the egg. (Approximately 224 cells present.)*
18	The cells have reached the egg-periphery and form small groups. (Approximately 872 cells present.)*
21	The cells form larger groups than before, especially at the postero-ventral end of the egg. (Approximately 1431 cells present.)*
23	The primary epithelium is formed at the postero-ventral end of the egg. The cells have reached the anterior end of the egg.
28	Completion of the primary epithelium all round the egg with clear differentiation into embryonic and extra-embryonic regions.
30	Cells in the germ disk region divide rapidly, resulting in the temporary loss of its uni-layered condition. First ventral groove and yolk-cell membrane make their appearance.
34	First ventral groove and yolk-cell membrane have disappeared by now. Beginning of degeneration of other yolk cell nuclei also. Many-layered condition of the germ disk persists.

* These figures are based upon actual counts made on individual eggs.

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TABLE I—continued

Age of egg (in hours after egg-laying)	State of development
42	Germ band differentiated into protocephalon and protocorm. Beginning of the differentiation of the inner layer (from the roof of the second ventral groove) at the cephalic end of the embryo. Formation of the cephalic fold of the embryonic membranes.
46	Region of inner layer differentiation has extended to the caudal extremity of the embryo. Appearance of the caudal and lateral folds of the embryonic membranes.
50	Completion of the embryonic membranes *i.e.*, closure of the amniotic cavity. Considerable elongation of the protocorm. Formation of head lobes. Beginning of primary segmentation of the inner layer. Stomodaeal rudiment appears.
Slightly older than 50	Primary external segmentation of the embryo into four segments and a corresponding segmentation of the inner layer. Appearance of antennary rudiments.
52	Paired rudiments of the labrum, jaw and thoracic appendages appear. Stomodaeal invagination formed.
53	Definitive external segmentation of the entire body except the abdomen. Rudiments of the first abdominal appendages appear.
56	Coelomic cavities of the head and thorax complete. Suboesophageal body differentiated.
59	Embryo is very long and thin. Provisional dorsal closure formed and thus epineural sinus arises. Proctodaeum formed. Neuroblasts of the brain differentiated. Optic lobes begin to be delaminated.
64	Neuroblasts of ventral nerve chain differentiated.
75	Definitive external and internal segmentation of abdomen complete. Coelom formation (except that of intercalary segment) complete.

2—*Cleavage and the Formation of the Primary Epithelium**

In the present paper the process of fertilization is not dealt with. McNABB (1928) has investigated maturation and fertilization stages in the Acridids *Chrotophaga viridifasciata* and *Circotettix verruculatus*. Recently, SLIFER and KING (1934) have studied the maturation divisions in fertilized eggs of *Melanoplus differentialis*, and KING and SLIFER (1934) in unfertilized eggs of the same insect. It is probable that these processes in *Locusta migratoria* are similar to those described in the above-mentioned Acridids. The earliest cleavage stage studied is the 4-cell stage (fig. 1*a*, *b*) which occurs at about $5\frac{1}{2}$ hours after egg-laying. These cleavage cells consist of large, stellate, protoplasmic masses with well-defined central nuclei. They measure, exclusive of the protoplasmic processes, about 16 μ in diameter. So far as could be determined, these cleavage cells are not connected with one another by protoplasmic strands. They cannot, therefore, be said to form a syncytium but are definitive cells. This is in contrast to some other insects where

* The term " primary epithelium " is equivalent to the blastoderm of most authors, the epiblast of GRABER (1891, *a*) and the Oberflächenepithel of HIRSCHLER (1924). The reasons for its adoption are discussed on p. 409.

up to the 8-nucleate stage definitive cleavage cells are not recognizable. Indeed, in some insects, it is the cleavage nuclei (not cells) which migrate to the egg-periphery, where the periplasm of the egg provides the cytoplasm round them. All the four cleavage cells lie very close to the posterior end of the egg, the furthermost cell being only about 495 μ from the posterior pole. The cells lie distinctly away from the periphery.

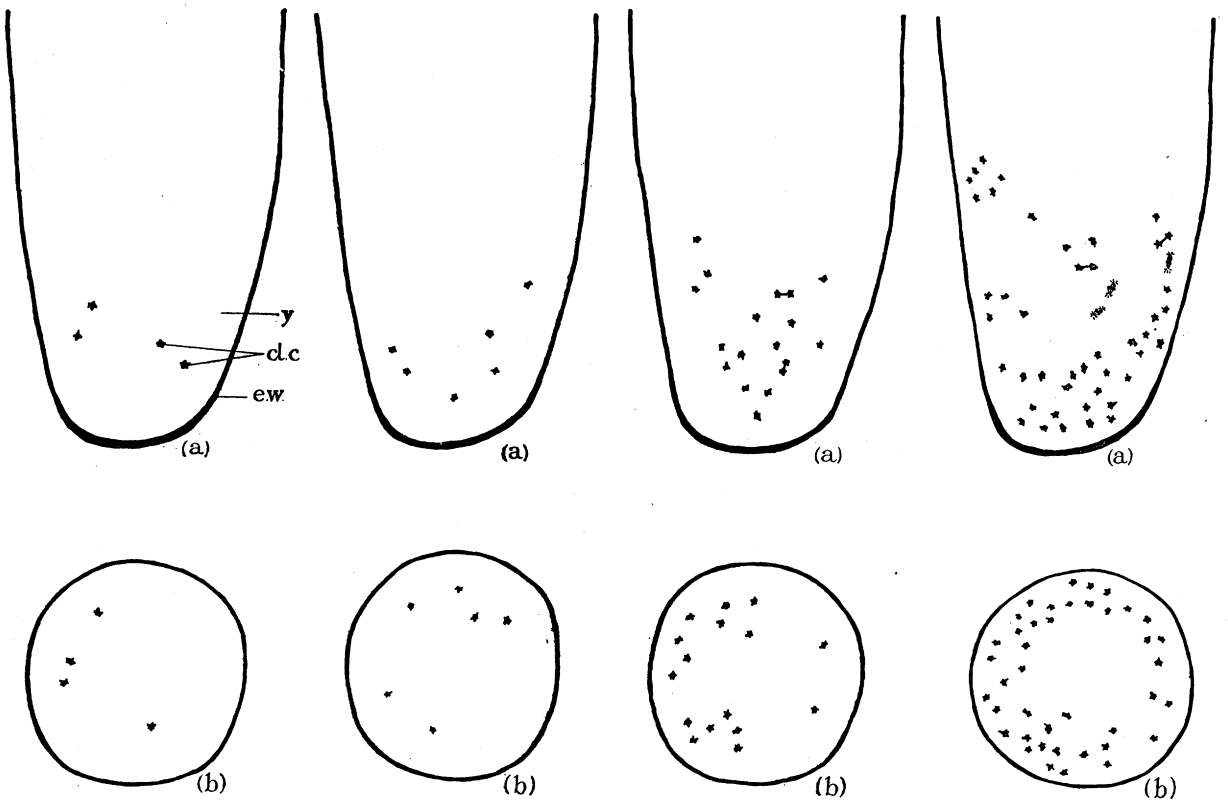

Fig. 1. Fig. 2. Fig. 3. Fig. 4.

FIGS. 1–4—Posterior regions of eggs, showing various stages of early cleavage. The figures have been drawn as sections with all the cells projected in a single plane. × about 27. (*a*) Longitudinal sections of posterior regions of eggs. (*b*) Transverse sections near posterior poles of eggs. *cl. c.*, cleavage cells ; *e.w.*, egg-wall ; *y.*, yolk.

FIG. 1—5½ hours old. 4 cleavage cells.
FIG. 2—7 hours old. 6 cleavage cells.
FIG. 3—8 hours old. 16 cleavage cells.
FIG. 4—10¼ hours old. 45 cleavage cells in (*a*) and 41 in (*b*).

At about the 7 hour stage (fig. 2*a*, *b*) six cells are seen. They have begun to migrate anteriorly, a process which becomes more and more pronounced as cleavage progresses. The farthest cell at this stage is about 560 μ from the posterior pole.

At the 8 hour stage (fig. 3*a*, *b*) already 16 cells are present, two of which are seen dividing (fig. 3*a*), being probably in the late telophase stage. The farthest cleavage

cell is about 760 μ from the posterior pole. The division rate, which was rather slow until the 7 hour stage, has now rapidly increased.

At the $10\frac{1}{4}$ hour stage (fig. 4*a*, *b*) about 41–45 cells could be counted, the deepest cell being about 1066 μ from the posterior pole of the egg. The cells have now started to migrate towards the egg-periphery. The disposition of the division spindles in these early stages do not show any definite orientation to the egg-periphery, a fact which is in accord with what has been described in the majority of insects so far studied.

All the cleavage cells are stellate but none of them shows comet-like protoplasmic extensions streaming behind the migrating cells (*cf.* Eastham, 1927 ; Sehl, 1931 ; and others). In the present case, therefore, the migration of the cleavage cells to the egg-periphery should be described not as a nuclear movement with the dragging of the cytoplasm behind the nucleus, but as a movement of the entire cell. In other words, the impetus of migration is shared equally by the nucleus and the cytoplasm. The cause of the migration has been ascribed by several workers to amoeboid movement. In the locust eggs, however, there is no evidence of such a movement, and Patten's (1884) idea, also accepted by Eastham (1927), of some centrifugal influence propelling the cells towards the periphery seems more acceptable.

At the 13 hour stage (fig. 5*a* and fig. 17, Plate 33) the cells are close to the egg-periphery. Their nuclei are rounded and the cytoplasm no longer shows stellate processes except in the cells left behind as primary yolk cells. The extremely fine periplasm can be seen only with difficulty. Vertically the cells have reached about half-way up the egg. From now onwards this vertical migration occurs along the egg-periphery and not through the yolk (fig. 7).

By the 18 hour stage (figs. 18 and 19, Plate 33) the cells have reached the egg periphery where they flatten out temporarily. This flattening of the cells immediately on reaching the egg-periphery is very probably a surface tension phenomenon. Such cells consist of a flattened nucleus with a thin coating of cytoplasm. They also show a tendency towards grouping. The yolk in the immediate neighbourhood of these cells is seen to be divided into very fine particles which are probably in a phase of digestion.

By the 21 hour stage (fig. 5*b* and fig. 20, Plate 33) the cells have migrated still further anteriorly but have not yet reached the anterior pole. The tendency towards grouping of cells, evident in the 18 hour stage, is here still more marked, especially at the postero-ventral end (fig. 39, Plate 35).

At about the 23 hour stage (fig. 5*c* and figs. 21 and 22, Plate 33) the cells at the posterior end of the egg are in a continuous layer, the primary epithelium, which forms the cup-shaped germ disk where the nuclei are arranged more or less regularly without forming any special groupings (*cf.* fig. 40, Plate 35). The cells on the rest of the egg-periphery do not as yet form a continuous layer. Those cells of the primary epithelium which form the germ disk are somewhat spindle-shaped, being elongated along the egg-periphery. Their nuclei are elongate-oval. The other peripheral cells are more rounded and so are their nuclei (fig. 23, Plate 33).

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The mode of division of the primary epithelial cells is mitotic. The division spindles are tangential to the surface of the egg and consequently cell division occurs at right angles to the periphery.

At about the 28 hour stage (figs. 25 and 26*a*, *b*, Plate 33) the primary epithelium forms a continuous layer all over the surface of the egg. The cells of the embryonic or germ disk region of the primary epithelium are closely packed together. They are columnar, being elongated perpendicularly to the egg-periphery, and have rounded nuclei. On the other hand, the cells of the extra-embryonic region of the

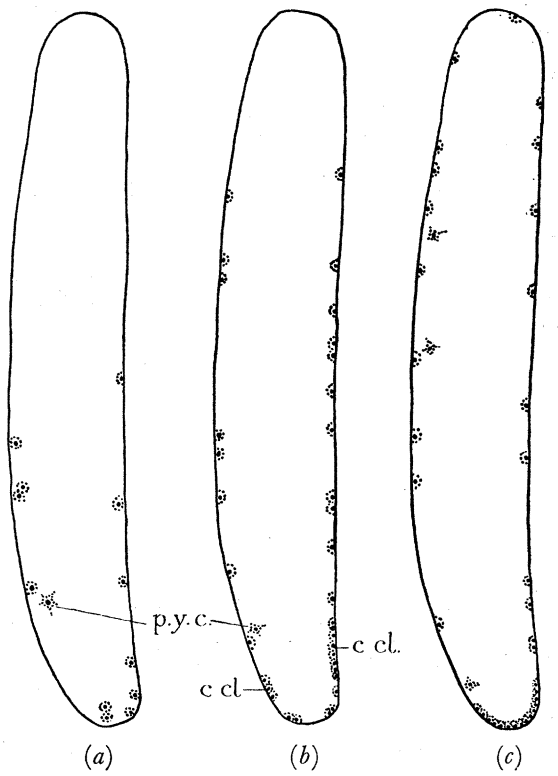

FIG. 5—Longitudinal sections of eggs, showing the migration of cleavage cells. Semi-diagrammatic. × about 16. (*a*) Egg 13 hours old ; (*b*) egg 21 hours old ; (*c*) egg 23 hours old. *c.cl.*, cell clusters ; *p.y.c.*, primary yolk cells.

primary epithelium, as well as their nuclei, are elongated tangentially to the egg-periphery. At the junction of the two areas, cells of one kind gradually merge into those of the other.

Discussion on the Formation of the Primary Epithelium—As will be seen from the foregoing account, the formation of the primary epithelium in *Locusta migratoria* does not occur simultaneously all over the egg-periphery. At first the posterior half of the egg is reached by the migrating cells. Then, by migration along the egg-periphery, the anterior half is covered. The definitive primary epithelium which forms the germ band first appears at the postero-ventral end of the egg and from there this epithelium formation spreads all over the egg. In other Orthoptera and Dermaptera

the first appearance of the primary epithelium is sometimes simultaneous (*Forficula*, Heymons, 1895) sometimes localized. Thus in *Gryllus* and *Periplaneta* (Heymons, 1895) it first appears at the hinder pole of the egg and then proceeds forwards. In *Gryllotalpa* (Korotneff, 1885 ; Heymons, 1895 ; Nusbaum and Fulinski, 1909) it first appears on the ventral surface, leaving the dorsal surface uncovered. In *Carausius morosus* (Leuzinger, 1925) a somewhat peculiar condition obtains. The primary epithelium first appears at the hinder pole of the egg, but the cleavage cells reach the neighbourhood of the anterior end only very late, viz., when the germ disk has already differentiated into the protocephalon and the protocorm ; further, these latter cleavage cells are very sluggish and do not divide. The anterior egg-pole itself remains entirely devoid of cells. Such a localized appearance of the primary epithelium has been noted in various insects, for instance, in *Hydrophilus* (Heider, 1889), *Donacia* (Hirschler, 1909), *Chalicodoma* (Carrière and Bürger, 1897), *Pieris* (Eastham, 1927), *Ephestia* (Sehl, 1931) and in many others. According to

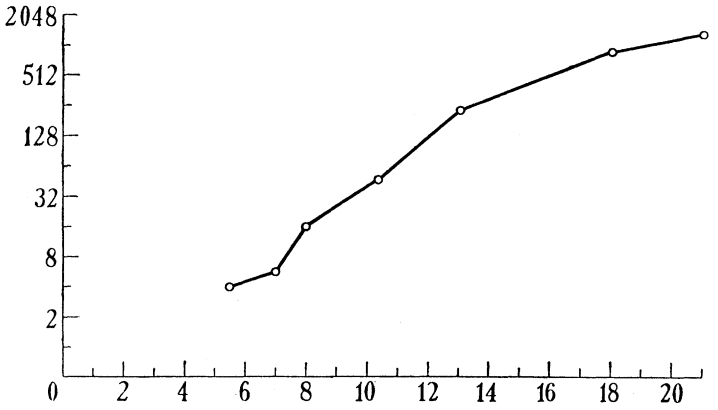

Fig. 6—Graphic representation of the time relations of early cleavage stages until the 21 hour stage.

Hirschler (1924), in spherical or slightly oval eggs, as for example those of *Forficula* and *Campodea*, the primary epithelium appears simultaneously on the entire surface of the egg. In elongated eggs it develops at different places of the egg surface at different times. This classification, however, cannot be regarded as a rigid one because in *Carausius* (Leuzinger, 1925), for instance, the egg is spherical, yet the primary epithelium appears first at the hinder pole.

Division and Migration Rate of Cleavage Cells—The approximate number of cleavage cells (including vitellophages) and of vitellophages recorded during early development is shown in Table II.

Table II

Cleavage Cells (Including Vitellophages)

Hours	$5\frac{1}{2}$	7	8	$10\frac{1}{4}$	13	18	21
Number	4	6	16–18	41–45	224	872	1431

Vitellophages

Hours	13	18	21
Number	47	45	36

In fig. 6, these results are expressed graphically. It is seen that the division is most rapid during the 7 to 10 hour period, after which there is a progressive slowing down. The period of greatest division activity thus coincides with a period during which the cleavage cells manifest no other activity. As soon as the cells begin to migrate towards the egg-periphery (at about the $10\frac{1}{4}$ hour stage), the division rate slows down. This conclusion agrees with similar results obtained by SEIDEL (1929) in the Libellulid *Platycnemis pennipes* and by SEHL (1931) in the moth *Ephestia kuehniella* ZELL.

As already mentioned, the migration of the cleavage cells towards the egg-periphery is first evident at about the $10\frac{1}{4}$ hour stage. After about the 13 hour stage, all the cells except the vitellophages have nearly reached the egg-periphery. Since both the distance travelled by these cells and the period during which this is accomplished are short, it is not possible to measure the rate of this migration. However, regarding vertical migration, *i.e.*, towards the anterior pole of the egg, the measurement of the migration rate is possible. Even during early cleavage stages when all the cells are more or less clustered together at the posterior pole of the egg, forming a sort of cylinder (fig. 4*b*), short but definite migration forward occurs. For the sake of convenience, this migration has been measured as the distance of the farthest cleavage cells from the posterior pole of the egg. From about the 13 hour stage onwards, this vertical migration is accomplished along the egg-periphery (fig. 7). At the 13 hour stage nearly half the length has been traversed and by about the 23 hour stage the anterior end of the egg is reached. During early development the approximate distance of the farthest cleavage cell from the posterior pole of the egg is shown in Table III.

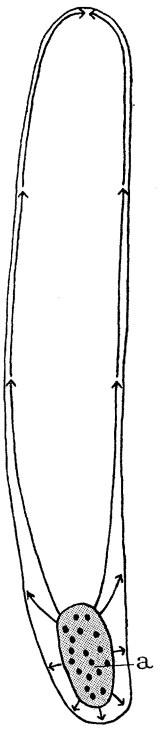

FIG. 7—Diagrammatic representation of the course of migration of cleavage cells after the 8 hour stage. (*a*) represents the extent of distribution of cleavage cells at the 8 hour stage. The arrows indicate the approximate paths of the cleavage cells.

TABLE III

Hours . . .	$5\frac{1}{2}$	7	8	$10\frac{1}{4}$	13	21	$22\frac{1}{2}$
Distance in μ	495	560	760	1066	2720	4400	5600

It is interesting to compare the migration rate of the cleavage cells with their division rate. At about the $10\frac{1}{4}$ hour stage when the rate of migration rapidly rises, the division rate rapidly falls. SEIDEL (1932) showed that in *Platycnemis* even the early cleavage nuclei undergo considerable migration, but in a definite manner. In *Locusta migratoria* such migration evidently does not occur. SEHL (1931) found that in *Ephestia kuehniella* the cells remain in a mass in the anterior third of the egg until the 32-cell stage. Afterwards they migrate towards the egg-centre, reaching the egg-periphery in about the 512-cell stage.

3—*First Ventral Groove and Yolk-Cell Membrane*

At about the 30 hour stage (fig. 27, Plate 33) it is seen that the cells of the germ disk epithelium are undergoing rapid division. This results in the epithelium temporarily losing its uni-layered condition and acquiring an irregular two or even three layered disposition of the nuclei at places. Such a condition has also been described in the Phasmid *Carausius morosus* (Leuzinger, 1925). At this stage there appear two extremely interesting phenomena which are important firstly, because they have been so far described only in one or two other insects; and secondly, because of their considerable theoretical significance in regard to the process of gastrulation and the origin of the primary endoderm among insects. They are described below.

First Ventral Groove (fig. 8 and figs. 27 and 28, Plate 33, and fig. 29, Plate 34)—At the above-mentioned stage there is seen, in the mid-ventral line of the germ disk and lying near the future cephalic end, a shallow, elongated groove on the outer face of the epithelium. It is about 88 μ long, 24 μ wide, and only 6 μ deep. From its roof several rounded cells are seen proliferating. These cells have nothing to do with the formation of the inner layer and very probably form secondary yolk cells. The whole appearance of the groove, combined with the proliferation of rounded cells from its roof, gives it the appearance of the so-called gastral groove. From this, however, it is to be distinguished since the latter makes its appearance some time afterwards, and has been termed in this paper as the "second ventral groove" in contradistinction to the "first ventral groove" described herewith. The first ventral groove lasts for about four hours or less. Nelsen (1934, *b*), while describing the process of "gastrulation" in the Acridid *Melanoplus differentialis*, makes no mention of the first ventral groove. However, it is evident from his figures (his Plate 1, fig. 1, and Plate 3, figs 17–21) that the condition represented in them corresponds to the first ventral groove (coupled with the many-layered condition of the germ disk which he has confused with inner layer formation).

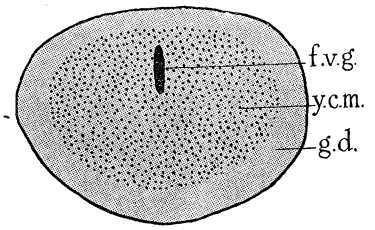

Fig. 8—Diagrammatic representation of the germ disk about 28 hours old, showing the first ventral groove and the yolk-cell membrane. Reconstructed from transverse sections. *g.d.*, germ disk; *f.v.g.*, first ventral groove; *y.c.m.*, yolk cell membrane.

Such a first ventral groove, as occurs in *Locusta migratoria*, has been recently reported in two other insects, viz., in *Pieris rapae* (Lepidoptera) by Eastham (1927) and in *Calandra granaria* (Coleoptera) by Inkmann (1933). In *Pieris* it lies near the cephalic end of the embryo on the ventral side and cells are proliferated from its roof as well as from a mid-ventral line behind the groove. These cells have been shown by Eastham to degenerate. The second ventral (gastral) groove of *Pieris* appears afterwards and is continuous with the first one. In *Calandra granaria* this groove (or "Ventralrinne" as Inkmann calls it in contrast to the later appearing "Primitiverinne" or second ventral groove) is short-lived as in *Locusta migratoria* and is not transformed directly into the second ventral groove. In *Gryllotalpa*,

KOROTNEFF (1885) found that although no "gastral" groove is formed, there occurs, soon after the completion of inner layer differentiation, a shallow, ventral, groove-like invagination which, however, bears no relation to the differentiation of the inner layer. So far as I am aware, there are no other references in literature to such "extra-gastral" invaginations. Their theoretical significance is discussed under "gastrulation" (p. 408).

Yolk-Cell Membrane (fig. 8 and fig. 27, Plate 33, and fig. 29, Plate 34)—Simultaneously with the appearance of the first ventral groove it is also seen that the yolk cells lying immediately beneath the germ disk are arranged in a layer. No cell boundaries are visible and the nuclei are connected by protoplasmic strands so as to form a continuous membrane of a syncytial nature. It meets the germ disk some distance inwards from the edge of the latter and extends beneath it as shown in fig. 8. By the 34 hour stage it has degenerated and no trace of it can be seen.

Such a yolk-cell membrane was first described among insects by HAMMERSCHMIDT (1910) in the Phasmid *Carausius* (*Dixippus*). He showed that, like the rest of the yolk cells, it degenerates. LEUZINGER (1925) and LEUZINGER and WIESMANN (1925) confirmed this in the same insect and further showed that this membrane takes some share in the formation of the mid-gut epithelium. These authors regard it as representing the primary endoderm which "fallen, . . . nachdem sie ihre Aufgabe als primäres, vorläufiges Mitteldarmepithel erfüllt haben, der Degeneration, der Auflösung anheim" (LEUZINGER and WIESMANN, 1925, p. 102). Thus, while in *Locusta migratoria* this membrane is extremely short-lived and its degeneration starts simultaneously with the first indication of degeneration of the other yolk cells, in *Carausius* this occurs some considerable time afterwards. The two structures are, however, homologous and represent a part of the primary endoderm. HEYMONS (1901) found such a membrane in *Scolopendra* where, however, it is not restricted to the germ band region but covers the entire yolk. EASTHAM's (1927) "limiting membrane to the yolk" may also belong to this category.

4—*Second Ventral Groove and Differentiation of Inner Layer*

At about the 34 hour stage (fig. 31, Plate 34) the germ disk is more markedly many-layered than before, although the cells do not exhibit any regular arrangement. Indeed, it would perhaps be more correct to speak of the germ disk as a syncytium. This condition is only temporary and disappears before the differentiation of the inner layer begins, so that this latter process takes place in an uni-layered germ disk. A multi-layered and syncytial early germ disk has been described in several other Orthoptera, viz., in *Carausius* (HAMMERSCHMIDT, 1910; and LEUZINGER, 1925), *Gryllotalpa* (HEYMONS, 1895) and in others. In these insects, however, there is no second ventral (gastral) groove and the multi-layered condition is intimately connected with the differentiation of the inner layer. Thus, LEUZINGER says: "Die Bildung der beiden primären Keimblätter erfolgt bei *Carausius* . . . gleichzeitig mit der Blastoderm- und Keimstreifbildung", although "Die Zellen der beiden Blätter können voneinander nicht unterschieden werden".

At about the 42 hour stage (fig. 42, Plate 35) the inner layer (the so-called "unteres Blatt", hypoblast, mesoderm or endo-mesoderm) is seen to arise as a proliferation of cells from the roof of a deep median-ventral groove. This groove, which I have termed the "second ventral groove" (*vide* discussion below), first makes its appearance at the cephalic end of the embryo a short distance from the extreme edge of the germ band. It extends rapidly towards the caudal end, and finally runs almost along the entire length of the embryo with the exception of the extreme cephalic end (figs. 38 and 43, Plate 35). In fig. 33, Plate 34, this groove is shown in section. The cells and nuclei of the inner layer, as well as those ectodermal cells and their nuclei which lie in the immediate neighbourhood of the second ventral groove, are rounded and smaller than the rest of the ectodermal cells and nuclei. The latter are columnar and their nuclei are oval. They are elongated at right angles to the egg-periphery. The second ventral groove is deep and well-marked. It lasts only for about three to four hours and is not met with after about the 46 hour stage. Fig. 38, Plate 35, which is a median-vertical longitudinal section of the posterior end of an egg 46 hours old, shows that the inner layer extends as a continuous layer from the caudal end of the embryo to a little distance behind the extreme cephalic end. It is, however, not of uniform thickness throughout its length, being much thicker at the caudal than at the cephalic extremity (also *cf.* figs. 34–36, Plate 34).

Graber (1888, *b*, and 1891, *a*) had previously observed a similar mode of inner layer formation in the Acridid *Stenobothrus variabilis* Fieb. More recently, Nelsen (1934, *b*) has described it in *Melanoplus differentialis* although, as pointed out above, he has failed to distinguish between the first and second ventral grooves. His remark that the "inner germ-band layer is formed by invagination, middle-plate formation and cell-proliferation" is apparently not correct. Inner layer formation in the Acrididae, as known from the examples mentioned above, occurs only by means of cell-proliferation from the roof of a mid-ventral, longitudinal invagination, here termed the second ventral groove.

Soon after its origin, the irregular mass of the inner layer (fig. 33, Plate 34) becomes wedge-shaped and fits into a similar notch in the germ band (figs. 34–36, Plate 34). The ectoderm now becomes more than one layered at places and the lateral halves of the germ band are thicker in the middle than at the sides. Shortly after the formation of the second ventral groove, the ectoderm bordering it in the cephalic region appears to be thickened into a pair of short, elongate swellings. These swellings are temporary and soon disappear (fig. 43, Plate 35).

Discussion—The mode of the formation of the inner layer among the Orthoptera is very varied but may be classed under either of these two principal heads, viz., origin by (*a*) invagination and (*b*) immigration. Table IV summarizes our present knowledge of inner layer formation in the Orthoptera and the Dermaptera.

It will be seen from Table IV how varied is the method of inner layer formation even in the closely related genera of the Orthoptera. All the three members of the family Acrididae so far studied, viz., *Stenobothrus variabilis*, *Melanoplus differentialis*,

TABLE IV—INNER LAYER FORMATION IN THE ORTHOPTERA AND THE DERMAPTERA

a. INVAGINATION (proliferation from the roof of a mid-ventral groove—the second ventral groove).	*b.* IMMIGRATION (proliferation occurs either (i) all over the germ band or (ii) mostly from special lateral areas of the germ band ; not from the roof of a groove).
ACRIDIDAE *Stenobothrus variabilis* (GRABER, 1888, *b*, 1891, *a*). *Melanoplus differentialis* (NELSEN, 1934, *b*). *Locusta migratoria migratorioides* R. *and* F. (ROONWAL—present paper).	PHASMIDAE *Carausius* (*Dixippus*) *morosus* (HAMMERSCHMIDT, 1910 ; STRINDBERG, 1914 ; LEUZINGER, 1925).
BLATTIDAE *Blatella* (*Phyllodromia*) *germanica* (WHEELER, 1889 ; CHOLODKOWSKY, 1891. But *cf.* HEYMONS, 1895 ; and NUSBAUM and FULINSKI, 1906). *Periplaneta orientalis* (HEYMONS, 1895).	BLATTIDAE *Blatella* (*Phyllodromia*) *germanica* (HEYMONS, 1895 ; NUSBAUM and FULINSKI, 1906. But *cf.* WHEELER, 1889 ; and CHOLODKOWSKY, 1891).
GRYLLIDAE *Oecanthus* (AYERS, 1884. Groove very shallow). *Gryllus* (WHEELER, 1893 ; HEYMONS, 1895).	GRYLLIDAE *Gryllotalpa* (KOROTNEFF, 1885 ; HEYMONS, 1895 ; NUSBAUM and FULINSKI, 1909).
MANTIDAE *Mantis* (GRABER, 1878 ; BRUCE, 1887 ; VIALLANES, 1891). *Stagmomantis* (WHEELER, 1893).	FORFICULIDAE *Forficula* (HEYMONS, 1895. Mostly from lateral borders of germ band).
TETTIGONIIDAE *Xiphidium* (WHEELER, 1893).	

and *Locusta migratoria migratorioides*, possess a deep, ventral, groove-like invagination extending almost throughout the entire length of the embryo and from whose roof the inner layer is proliferated. Of the other families, some show only one, while others show both modes of inner layer formation as shown below :—

(*a*). Inner layer formed only through invagination.	(*b*). Inner layer formed only by diffuse or by localized immigration.
Acrididae. Tettigoniidae. Mantidae.	Phasmidae.

(*c*). Inner layer formed by both modes, (*a*) and (*b*).

Blattidae.
Gryllidae.

The above grouping is only tentative because, although in some insects the presence or absence of the second ventral groove has been demonstrated beyond doubt, in others the evidence is either conflicting (as, for instance, in *Blatella* (*Phyllodromia*) *germanica*) or is otherwise unsatisfactory, being based on faulty technique, so as to demand re-investigation. In still others the number of representatives studied from a particular family is only either one or two.

In *Locusta migratoria* the differentiation of the inner layer occurs from a single area, viz., from an elongated median-longitudinal line, that is to say, its developmental mode is unitary (to use the term of HIRSCHLER, 1924) and localized. Such a unitary mode of inner layer formation has been described by WHEELER (1889) in *Blatella* (*Phyllodromia*) *germanica* where the inner layer arises as a small area at the hind end of the germ band and progresses forwards. This condition is contrary to that which occurs in *Locusta migratoria*, where the differentiation of this layer proceeds from the cephalic towards the caudal end. On the other hand, in some other Orthoptera the inner layer develops in the beginning as two or more well-marked patches which grow towards each other and unite, thus acquiring a unitary character only secondarily. This occurs, for example, in *Gryllotalpa* (NUSBAUM and FULINSKI, 1909) where the inner layer arises from four separate rudiments. With regard to the area which the inner layer at the time of its formation occupies in relation to the entire extent of the germ band, two extremes can be distinguished among insects. In one the inner layer is restricted to a small area of the germ band, *i.e.*, it is localized. This occurs in *Lepisma* (HEYMONS, 1897, *a*). In the other, the inner layer extends almost over the entire surface of the primary epithelium, in other words, it is diffuse. This condition obtains in *Isotoma* (PHILIPTSCHENKO, 1912, among the Apterygota ; among the Pterygota it occurs in some Orthoptera, viz., *Blatella* (*Phyllodromia*) *germanica* (HEYMONS, 1895), and *Gryllotalpa* (HEYMONS, 1895 ; NUSBAUM and FULINSKI, 1909), and in the Isopteran genus *Eutermes* (KNOWER, 1900). The majority of the other insects, including *Locusta migratoria*, occupy a position mid-way between these two extremes. It should be pointed out that the presence of a localized, or of a diffuse, mode of inner layer formation does not appear to have any phylogenetic significance. The Apterygota show both the modes and so do the Pterygota.

5—*Yolk and Yolk Cells*

Yolk—The structure of the yolk has been studied in sections cut by HEIDER's method (1889) of painting with collodion. At the time of oviposition, the yolk of *Locusta migratoria* is like a viscid fluid composed of large and small yolk spheres, with minute droplets of fat scattered throughout the whole egg. The yolk spheres have a characteristic distribution. Except at the poles and the egg-periphery, they are large and more or less rounded. Near the poles, however, they become smaller, giving the yolk a granular appearance. All round the egg-periphery a thin layer of yolk composed of very minute particles can be distinctly seen. The protoplasmic reticulum is not distinct in the early stages, but, when the amount of yolk is diminished

in older stages, it becomes evident. In *Blatella* (*Phyllodromia*) *germanica* (PATTEN, 1884) a similar distribution of yolk occurs, with the difference that the granular yolk is not specially abundant at the poles. The peculiar formation of two zones described in the same insect by BLOCHMANN (1887) does not occur in *Locusta migratoria*. The yolk spheres are at first closely packed together. As the egg grows older, they become sparsely distributed. At the same time, the yolk mass is pushed towards the anterior end of the egg, the posterior end being occupied by the embryo. Also, evidently as a result of assimilation, the yolk changes in character. This is shown by its greater ease and less brittleness in cutting in the older stages than in the younger ones. The yolk is also less viscid than formerly.

Secondary Yolk Cleavage—The most important change that the yolk undergoes, from the morphological point of view, is the so-called secondary yolk cleavage (fig. 9). At about the 60 hour stage the yolk, which has so far remained "amorphous" and taken no share whatever in the original cleavage processes, begins to show polyhedral differentiation in the immediate neighbourhood of the embryo. This feature spreads to the rest of the yolk in a postero-anterior direction. By the 75 hour stage, the entire yolk becomes divided into polyhedral masses, which can be seen externally even in the living egg. This phenomenon lasts for about 24 to 36 hours and then completely disappears so that in an egg about 100 hours old no trace of it can be seen. The yolk polyhedrals are sharply defined and each generally contains a large nucleus, although occasionally enucleate examples are found.

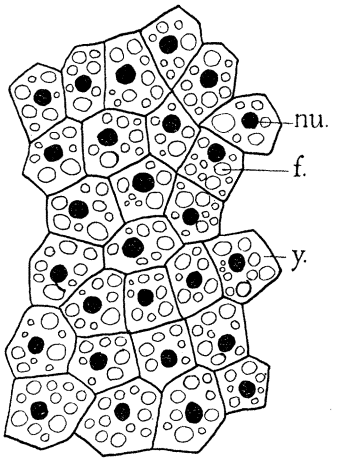

FIG. 9—A portion of the yolk from an egg 70 hours old, showing secondary yolk cleavage (semi-diagrammatic). × about 75. *f*, negative images of fat globules; *nu*, nucleus; *y*, yolk.

This transient phenomenon of secondary yolk cleavage is of considerable theoretical importance from the point of view of comparative morphology. It has been described in the Orthoptera, the Dermaptera, the Coleoptera, and the Lepidoptera and is termed "secondary" in contradistinction to "primary yolk cleavage" occurring in the majority of the Apterygota and in some parasitic Hymenoptera and the Strepsiptera. The ant *Azteca* (STRINDBERG, 1916) is of special interest since it is the only non-parasitic Pterygotan in which a total cleavage (and thus a primary yolk segmentation) occurs. STRINDBERG (1913–19) has also described in several other ants (*Camponotus*, *Leptothorax*, *Formica*) an early partial cleavage of the superficial ("sub-blastodermal") yolk, resulting in the formation of yolk pyramids—a phenomenon partly comparable to total cleavage. It is interesting to compare these yolk pyramids with those occurring in the other Arthropoda. Thus, they occur in several Myriapods (HEYMONS, 1901, *Scolopendra*; and others), Crustacea (REICHENBACH, 1886, both primary and secondary yolk pyramids; MANTON, 1928, *Hemimysis*, secondary yolk pyramids; and others), Arachnids (MORIN, 1886,

Theridium ; and others), Pantapoda (MORGAN, 1891 ; and others), and finally the Onychophora (SHELDON, 1888, *Peripatus novaezaelandiae*). This, and similar evidence, which need not be marshalled here, point towards the fact, as suggested by STRINDBERG, that the secondary yolk cleavage is only a belated expression of true cleavage activity, and the yolk polyhedra are comparable to true yolk cells. With this view I am in complete agreement. What share then do these yolk cells, or, in some cases, yolk syncytia, take in the further development of the embryo ? For the majority of insects the older view of the origin of the mid-gut epithelium from yolk cells is no longer held. But in *Lepisma* and *Campodea* (HEYMONS, 1897, *a*, *b*), in Libellulids (TSCHUPROFF, 1903), and in a few other insects the definite origin of the mid-gut epithelium from yolk cells has been shown. Of special interest is TSCHUPROFF's observation that in the Libellulidae only the middle part of the mid-gut epithelium is of yolk cell origin, the rest being ectodermal. This suggests the gradual obliteration of the yolk cell element from the mid-gut of higher insects. In the majority of the primitive Pterygota, the yolk cells, after their sudden and belated outburst of activity in the form of secondary yolk cleavage, soon lose all morphogenetic value and degenerate. In some other Pterygota, even this expression of activity has been suppressed.

Yolk Cells—In *Locusta migratoria* not all the cleavage cells reach the egg-periphery to form the primary epithelium. Some remain beneath this layer and form the so-called vitellophages or primary yolk cells. In other words, " intra-vitelline separation " of HEYMONS occurs. The primary yolk cells retain the appearance of early cleavage cells, *i.e.*, they are stellate and have large, round nuclei. During the early stages of intravitelline separation, all the yolk cells are more or less superficial in position. At about the 13 hour stage when the cleavage cells have nearly reached the egg-periphery, the number of primary yolk cells is about 47 out of a total of about 224 cleavage cells. It is possible that some of the former may, at this early stage, still represent tardy cleavage cells which have not reached the egg-periphery. Their number gradually decreases up to the 21 hour stage when there are only about 36 primary yolk cells out of a total of about 1431 cleavage cells. After this stage, however, there is a rapid increase in their number due to the formation of secondary yolk cells. Thus, at about the 23 hour stage, nearly 119 vitellophages could be counted, all lying near the periphery of the egg.

Our present knowledge of the presence or absence of an intravitelline separation among the Orthoptera is summarized in Table V.

The various ways by which the secondary yolk cells of *Locusta migratoria* arise are described below. Firstly, they may arise by migration of primary epithelium cells. Individual cells become amoeboid and migrate centripetally from the egg-periphery (figs. 21 and 24, Plate 33). This mode is multipolar and not restricted to any special region of the egg-periphery. It was first recorded by WHEELER (1889) in *Blatella* and afterwards by HEYMONS (1895) in other Orthoptera and Dermaptera. SCHWANGART (1904 and 1906) classified insects into two groups, viz., those where such a migration is multipolar and those where it is localized, no intermediate

TABLE V—THE ORIGIN OF YOLK CELLS IN THE ORTHOPTERA

Intravitelline separation occurs— (primary yolk cells formed)	No intravitelline separation occurs— (no primary yolk cells formed)
ACRIDIDAE	PHASMIDAE
Locusta migratoria migratorioides R. and F. (ROONWAL—present paper).	*Carausius* (*Dixippus*) *morosus* (HAMMERSHMIDT, 1910 ; LEUZINGER, 1925).
GRYLLIDAE	GRYLLIDAE
Gryllus (HEYMONS, 1895). *Oecanthus* (AYERS, 1884). *Gryllotalpa* (NUSBAUM and FULINSKI, 1909. But *cf.* KOROTNEFF, 1885, and HEYMONS, 1895).	*Gryllotalpa* (KOROTNEFF, 1885, and HEYMONS, 1895. But *cf.* NUSBAUM and FULINSKI, 1909).
MANTIDAE	BLATTIDAE
Mantis (GIARDINA, 1897).	*Blatella* (*Phyllodromia*) *germanica* (WHEELER, 1889 ; HEYMONS, 1895). *Periplaneta* (WEISMANN, 1882) ; KOROTNEFF, 1885 ; HEYMONS, 1895).

It will be seen that among the Gryllidae both the modes occur.

condition occurring. This classification, however, is not acceptable because both modes may occur in one and the same insect, as for example, in *Calliphora* (NOACK, 1901). A second mode is by the division of the primary epithelium cells. Certain cells become amoeboid and divide. The inner product of such a division is interpreted as forming a secondary yolk cell (fig. 10*a*, *b*). This mode has not been described previously in any other insect. The origin of secondary yolk cells from primary yolk cells could not be ascertained as no division stages of the latter have been met with.

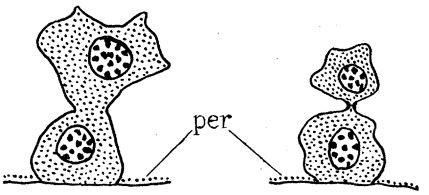

FIG. 10—Two late stages in the formation of a secondary yolk cell by division of a primary epithelium cell. From the posterior pole of an egg about 23 hours old. × 370. *a*, Early stage ; *b*, stage near completion of division. *per.*, periplasm.

The secondary yolk cells were observed undergoing division in very few instances only, and in these examples no mitotic stages could be detected. It is, therefore, not possible to state definitely which type of nuclear division (mitosis or amitosis) prevails. In other insects, however, these cells have been shown to divide both amitotically (SCHWARTZE, 1899 ; TOYAMA, 1902 ; MARSHALL and DRENHEHL, 1906 ; and others) and mitotically (NELSON, 1915 ; HUIE, 1917 ; EASTHAM, 1927 ; SEHL, 1931 ; and others).

In about the 28 hour stage the yolk cells immediately beneath the germ disk arrange themselves in a single layer. This layer, at about the 30 hour stage, is seen to form the yolk-cell membrane described above. Of the other yolk-cells which do not share in the formation of this membrane, some lie singly and are stellate, while others clump together into twos and threes or more to form irregular syncytia which do not

show any stellate processes (figs. 29 and 30, Plate 34). These yolk-cell syncytia become enlarged probably by amitotic division of their constituent nuclei. At the 34 hour stage, no trace of the yolk-cell membrane is seen—it has undergone complete degeneration. At this stage, also, some of the other nuclei show the onset of degenerative changes (fig. 31, Plate 34). This degeneration of yolk cells is, however, considerably slower than their reinforcement, so that their number continuously increases for a long time. The degenerative changes in a yolk cell occur in a manner similar to that described by WIESMANN (1926) in *Carausius*. At about the 40 hour stage the yolk-cell syncytia described above are seen to be still more enlarged and may contain seven or even more nuclei in each. Soon afterwards, however, they disappear and only single yolk cells are met with. All this time the yolk-cells have been penetrating deeper and deeper into the yolk and thus become distributed throughout the egg.

The morphological position of the primary and secondary yolk cells is discussed below.

6—*General Discussion on Gastrulation, with a New Theory of Multi-phased Gastrulation Among Insects*

a. General Discussion—The understanding of the nature of insect gastrulation is bound up firstly with the interpretation of the nature and morphological position of the yolk cells, and secondly with the mode of origin of the mid-gut epithelium. On the answer to these questions will naturally depend what process or processes in insect development are to be regarded as corresponding to gastrulation among other animals. In general, gastrulation is characterized by the following features :—(i) It is a process by which the single-layered embryo becomes two-layered, the ectoderm and the endoderm being thus laid down. Soon afterwards, the embryo becomes three-layered owing to the differentiation of the mesoderm from the endoderm. Thus, differentiation of the endoderm precedes that of the mesoderm. (ii) Gastrulation may occur by any of the following four processes or their combinations, viz., by emboly or invagination, by epiboly or overgrowth, by delamination, and lastly, by inward migration of cells from one pole of the gastrula. In alecithal eggs gastrulation is generally a simple, clear process, usually brought about by epiboly, and the usual sequence of events is neither much delayed nor much disturbed. On the other hand, in eggs which are rich in yolk, as for example insect eggs, the whole process of gastrulation becomes complicated owing largely to the fact that the presence of yolk tends to retard general cell-activity.

There exists to-day two main views regarding the nature of gastrulation among insects.

1. A true blastula stage occurs in insects and is formed when the cleavage cells reach the egg-periphery. Gastrulation occurs afterwards when the inner layer is differentiated. The yolk cells have no morphological significance so far as the germ layers are concerned. The endoderm generally arises as a bipolar structure—at

the blind ends of the stomodaeal and proctodaeal invaginations. This view was advanced by KOWALEWSKY (1886) and is supported by WHEELER, NUSBAUM, and FULINSKI, STRINDBERG (in a slightly modified form), PHILIPTSCHENKO, and others, and more recently by MANSOUR (1927), and EASTHAM (1927 and 1930).*

2. No true blastula stage occurs among insects. The so-called blastula is in reality a post-gastrula stage. The supporters of this view differ among themselves in certain points and their views can be classed into two groups, viz.,

(*a*) Older biphased gastrulation theory. This was first clearly put forward by HEYMONS (1895 and 1901). According to him, insect gastrulation occurs in two phases of which the first is represented by the separation of the primary yolk cells (intravitelline separation), and the second by the inward migration of secondary yolk cells from the primary epithelium (circumpolar separation). The differentiation of the inner layer is not regarded as a part of gastrulation. PATTEN was also of this opinion and says (1890, p. 368), " That the median furrow of insects is merely an ontogenic adaptation is sufficiently evident from the fact that it may be present or absent in closely related forms ". According to this view, the yolk cells alone represent the endoderm. But since they eventually degenerate, there is, among the Pterygote insects, no true endoderm sharing in the formation of the adult body. The mid-gut epithelium is of ectodermal origin. This view has been supported by LÉCAILLON (1897–8), SCHWARTZE (1899), and the majority of those authors who regard the mid-gut epithelium as ectodermal in origin.

(*b*) Newer biphased gastrulation theory. This view also maintains insect gastrulation to occur in two phases. The first phase corresponds to the first phase of the older view discussed above. But the second phase is represented here by the differentiation of the inner layer and the inwandering of the yolk cells from the primary epithelium. Thus, there is, among insects, no blastula stage and therefore no blastoderm. The primary epithelium (or Oberflächenepithel of HIRSCHLER, 1924) is equivalent to the ectoderm which is composed of two potential elements, viz., a provisional membrane epithelium which goes to form the transitory embryonic membranes, and a definitive germ band epithelium which forms the germ band proper. The latter is potentially more than ectoderm since from it there differentiates the inner layer (= endo-mesoderm). The yolk cells represent the provisional primary endoderm. The secondary endoderm, which arises from the inner layer at the blind ends of the stomodaeum and the proctodaeum, goes to form the mid-gut epithelium. This theory, first suggested by WILL (1888), has received support from a number of authors including NOACK (1901), DICKEL (1904), SCHWANGART (1904–1906), and HIRSCHLER (1912, 1924). Although agreeing in fundamentals, these authors differ from one another in details. Thus, SCHWANGART maintained that,

* HENSON (1932) has recently attempted to homologize the stomodaeal and proctodaeal invaginations of *Pieris* embryo with the oral and anal remnants of the blastopore of *Peripatus*. The blastopore of *Peripatus* is formed simultaneously with the differentiation of the endo-mesoderm. The stomodaeal and proctodaeal invaginations of *Pieris*, on the other hand, appear long after the differentiation of the endo-mesoderm (inner layer). HENSON's view, is, therefore, unacceptable.

in the Lepidoptera studied by him, the yolk cells take some share in the formation of the mid-gut epithelium. This conclusion, however, is not accepted by HIRSCHLER who worked on the same group of insects. Again, NOACK, DICKEL, and SCHWANGART believed that in the different groups of insects studied by them, viz., the Diptera, the Hymenoptera, and the Lepidoptera respectively, a shallow invagination occurred during the first gastrulation phase. This was denied by HIRSCHLER, according to whom a gastral groove does not exist in the first gastrulation phase. This latter author has discussed (1924) the newer biphased gastrulation theory in an excellent and convincing manner. He has shown that the primary yolk cells of insects are to be regarded as equivalent to the Annelidan macromeres. The most convincing evidence for this view we get from the embryology of the Strepsiptera (HOFFMANN, 1914; NOSKIEWICZ and POLUSZYNSKI, 1928), as discussed below. Further, in insects, all the three germ layers, viz., ecto-, meso-, and endoderm share in the formation of the completed body, although the share of the endoderm is considerably reduced and modified. This change of reduction and modification has been brought about by the large amount of yolk present in insect eggs. HIRSCHLER (1912) has shown that such modifications are not restricted to insect eggs alone, but also occur in other Arthropods and in various other animal groups, viz., fishes (Teleostians and Selachians), Cyclostomes, Tunicates, and Cephalopods. Phylogenetically, biphased gastrulation is a secondary feature brought about by the large amount of yolk. The process of intravitelline separation of yolk cells, on the other hand, is primary. Its absence is secondary, so that the non-existence of the first gastrulation phase in a few insects should be regarded as a secondary modification.

b. A New Theory of Multi-phased Gastrulation Among Insects—The theory to be discussed is essentially a development of the newer biphased gastrulation theory and has been necessitated by certain facts recently discovered by myself and other authors. It also attempts, in part, to combine the already described older and newer biphased gastrulation theories. From both of these, however, it differs in the fact that insect gastrulation is no longer to be regarded as a process occurring in only two main periods of activity but in several. This, it need hardly be pointed out, has been brought about by the large amount of yolk present in insect eggs. The whole process of gastrulation has been extremely elongated in time and the time relations of the various phases of the process are profoundly modified. The main theme of this theory will be developed below with reference to what occurs in *Locusta migratoria* and then compared with other insects. Gastrulation in *Locusta migratoria* occurs, according to this view, in the following stages :—

First Phase—Cleavage. Intravitelline separation. (This phase of gastrulation occurs by modified epiboly and results in the differentiation of the primary endoderm represented partly by the primary yolk cells.)

Second Phase—First ventral groove (corresponding to part of gastral groove). Yolk cell membrane (corresponding to part of evanescent primary endoderm). Multi-layered condition of the germ band is an indication of activity during this phase.

Third Phase—Second ventral groove (corresponding to part of gastral groove). Formation of inner layer (endo-mesoderm).

Fourth Phase—Secondary yolk cells (corresponding to part of secondary endoderm ; transient).

Thus, in *Locusta migratoria* there are four main periods of activity into which the whole process of gastrulation is divided. These periods are fairly sharply demarcated from one another, although they sometimes overlap, as for example in the formation of the secondary yolk cells which is spread over a long period. Nevertheless, one can roughly speak of gastrulation as occurring in four phases. The first, third and, to some extent, the fourth phase occur in all insects, but the second phase has, so far, been recorded in a few insects only. However, the phenomena occurring during this last phase have an important bearing on gastrulation. Evidence has recently accumulated which shows that these processes are by no means confined to *Locusta migratoria*. Thus, the presence of a yolk-cell membrane in *Carausius* (*Dixippus*) *morosus* (Hammerschmidt, 1910 ; Leuzinger, 1925 ; Leuzinger and Wiesmann, 1925), and of a first ventral groove in *Pieris rapae* (Eastham, 1927) and in *Calandra granaria* (Inkmann, 1933, " Ventralrinne ") lends support to the importance of this phase of gastrulation. The formation of a groove soon after the differentiation of the inner layer of *Gryllotalpa* (Korotneff, 1885) indicates the presence of a phase of gastrulation activity which falls between the third and fourth phases of *Locusta migratoria*. It is thus clear that insect gastrulation occurs in three or more main phases, their actual number varying in different insects. The term " multi-phased gastrulation " is, therefore, proposed to cover all these phases. Since it is very likely that more exact work on the early stages of insect embryology will bring to light structures such as supernumerary ventral grooves, etc., which may represent some phase of gastrulation, I have refrained from giving definite names to the various phases of gastrulation as observed in *Locusta migratoria*. The terms tentatively adopted depend on their time relations in this insect. Nevertheless, it has been considered necessary to make certain changes in the nomenclature of some structures formed during the early embryonic period of insects. I propose the adoption of the following terms (some of which are already in use) :—

Primary Epithelium (corresponding to the so-called blastoderm)—Its German equivalent " Oberflächenepithel " was first suggested by Hirschler (1924). Some authors still regard a true blastula to occur among insects and the primary epithelium as the blastoderm. The term " primary epithelium " is non-committal, and hence to be preferred.

Primary Yolk Cells—These are the cells that are left behind in the yolk as a result of intravitelline separation. They are a part of the primary endoderm and are to be compared to the Annelidan macromeres.

First Ventral Groove—Is part of the so-called gastral groove which is formed afterwards. Has been recorded in *Locusta migratoria*, *Pieris rapae*, and *Calandra granaria*. In *Pieris* it is continuous with the second ventral groove but in the other two insects it is not.

Second Ventral Groove—This is equivalent to the gastral groove of most authors and the mesodermal groove of Lécaillon (1898). Present in many insects but absent in others. Is part of the gastral groove.

Third Ventral Groove—This has been recorded by Korotneff (1885) in *Gryllotalpa*, and is formed after the differentiation of the inner layer. Hence it is not equivalent to the first and second ventral grooves but comes after them, although in *Gryllotalpa* both the first and second ventral grooves are absent.

Inner Layer—This is the layer which is differentiated from the primary epithelium on the inside, by any of the various methods of gastrulation. It is equivalent to the " unteres Blatt ", mesoderm, endo-mesoderm, hypoblast, etc., of other authors and is, potentially, an endo-mesoderm. Since some authors still regard it as pure mesoderm, the non-committal term " inner layer " is adopted here.

Secondary Yolk Cells—These are cells given off into the yolk after the first gastrulation phase, *i.e.*, after the formation of the primary yolk cells. They are part of the secondary endoderm.

The development of the mid-gut epithelium of *Locusta migratoria* has been studied by me and the full account will appear in the second paper of this series. It is shown to be ectodermal in origin. I have also discussed there the nature of the insect endoderm. Since a consideration of this question is of importance in dealing with gastrulation, I shall describe very briefly the conclusions I have arrived at. These are : (1) that the insect endoderm consists of primary and secondary portions (the primary and secondary endoderm of the German authors) ; (2) that the definitive insect mid-gut epithelium, no matter in what manner arising, is, in the majority of insects, a secondary phenomenon. It may arise from pure ectoderm, from the inner layer (secondary endoderm) or from the ectoderm plus secondary yolk cells (= secondary endoderm). In the Strepsiptera alone (Hoffmann, 1914 ; Noskiewicz and Poluszynski, 1928) do the *primary* yolk cells form a transient mid-gut (" primary mid-gut " of Hoffmann).

7—*The Formation of the Embryonic Membranes*

The formation of the embryonic membranes in *Locusta migratoria* begins almost simultaneously with the differentiation of the inner layer. It is brought about by the inward folding of the lateral borders of the germ band ventrally. At about the 42 hour stage there appears the cephalic fold of the embryonic membranes (figs. 32 and 34, Plate 34, and fig. 42, Plate 35). Afterwards, at about the 46 hour stage, a similar fold appears at the caudal end of the embryo (fig. 36, Plate 2, and fig. 43, Plate 35). Meanwhile, the region of the embryo lying between the cephalic and the caudal folds also begins to grow round and forms the lateral folds (fig. 35, Plate 34). The cephalic and the caudal folds travel towards each other and eventually fuse with the lateral folds which also close ventrally. At about the 50 hour stage, the formation of the embryonic membranes is complete. In the early stages of membrane formation, the inner membrane or amnion cannot be

clearly distinguished from the embryonic region (fig. 35, Plate 34). Later on, however, when the folds have fused together on the ventral side of the embryo, this distinction is possible. In this way, two embryonic membranes are formed, viz., the amnion and the serosa. The amnion is differentiated from the embryonic region and consists of elongated cells with elongate nuclei. The serosa is a continuation of the extra-embryonic portion of the primary epithelium and is composed of typical pavement cells which are polygonal in shape. Their nuclei, like those of the amnion, are disk-shaped, being flattened at right angles to the egg-periphery and present a lenticular appearance when viewed from the edge. They measure about 24–28 μ in diameter, and are generally irregularly distributed. At the posterior pole of the egg, however, (and later on at the anterior pole also), the serosal nuclei are closely crowded together to form a circular area of about 380 μ in diameter (fig. 37, Plate 34). In this area the nuclei decrease in size from the periphery towards the centre. The peripheral nuclei measure about 16 μ in diameter and the central ones about 10 μ or less. The two membranes are at first closely applied to each other (fig. 34, Plate 34). Soon, however, they separate at the sides and a thin layer of yolk comes to lie between them (fig. 36, Plate 34, and fig. 38, Plate 35). But in the centre they still remain closely applied to each other and no yolk is seen between them in this region.

With regard to the position of the embryo after membrane formation, the majority of insects fall into one of the two groups, viz., the superficial germ-band type and the submerged type. In the former, the amnion and the serosa are closely applied to each other so that there is no yolk in between them. In the latter, the two membranes are separated by a layer of yolk—the germ band is thus, so to speak, submerged into the yolk. The Acrididae occupy an intermediate position between these two types. In *Locusta migratoria* the amnion and the serosa are separated at the sides by a fairly thick layer of yolk which may even contain yolk cells. But in the middle, they are either closely applied to each other or are separated only by a very thin layer of fluid in which there are no yolk particles. Graber (1888, *a*, *b*) has described the same condition in another Acridid *Stenobothrus*. This intermediate condition has not been described in other insects.

8—*Changes in the External Form of the Embryo and the Primary Segmentation of the Inner Layer*

Graber (1888, *b*, 1890) gave a good account of the changes in the external form, etc., of the embryo of *Stenobothrus*. More recently, Nelsen (1931, 1934, *a*) and Slifer (1932, *a*) have described the external changes of form in the embryo of *Melanoplus differentialis*, but their accounts of the early stages are incomplete.

The germ disk of *Locusta migratoria* starts forming as a thickening of the primary epithelium cells lying at the posterior pole of the egg, slightly on the ventral side (fig. 25, Plate 33, and figs. 39–41, Plate 35). Thus, a round, concave germ disk arises which is composed of large, prismatic cells with small, disk-shaped nuclei

measuring approximately 8 μ in diameter. The cells of the extra-embryonic primary epithelium are more or less spindle-shaped. Their nuclei measure about 16 μ in diameter and are thus about twice the size of those of the embryonic cells The germ disk afterwards begins to elongate at its ventro-anterior end along the ventral side of the egg. In about the 42 hour stage, two distinct regions of the embryo can be distinguished—the broad protocephalon and the narrow, elongated protocorm (fig. 42, Plate 35). At this stage the two regions are nearly equal in length but differ in width. The protocephalon measures about 464 μ across, and the protocorm only about 250 μ. The latter tapers slightly at its extremity. The total length of the germ band, at this stage, is about 665 μ. (In exceptional cases, the protocorm may arise not from the edge of the germ disk as usual, but from its middle). A notch in the mid-frontal region of the embryo is seen at this stage, and is due to the sides of the embryo in this region developing faster than the middle. It, however, soon disappears.

The protocorm rapidly elongates and, in about the 46 hour stage is already about twice as long as the protocephalon (fig. 43, Plate 35). External body segmentation of the embryo has not yet begun, but the middle region of the protocorm shows, on either side, a slight swelling. These swellings, however, are of no segmental significance. The inner layer, at this stage, extends all along the germ band, except the extreme frontal region of the protocephalon.

In about the 50 hour stage (fig. 44, Plate 35) the inner layer begins to show signs of segmentation especially in the thoracic region. At the anterior end it is swollen and has a notch in front. The rudiment of the stomodaeum is also seen. The head lobes are greatly developed and are bent ventrally and medianally at the edges.

In a slightly older stage, the germ band is seen to have divided into four primary segments—a protocephalic and three protocormic elements. The inner layer has also undergone a segmentation into four parts which roughly correspond to the external primary segmentation. The antennary rudiments have made their appearance. No other appendages are seen as yet.

In a later stage, viz., the 52 hour stage (fig. 11), the rudiments of the three jaw and the three thoracic appendages are seen to have been differentiated in the inner layer and the latter is divided into two lateral halves in each of these segments. Externally, however, these segments are not yet established. The primary external segmentation is less distinct than before. Thus, the definitive segmentation of the inner layer precedes that of the ectoderm.

In the 53 hour stage (fig. 12), the definitive external segmentation of the body becomes evident. The abdomen is still unsegmented, although the rudiments of the first abdominal appendages are seen as a pair of thickenings of the inner layer. By about the 75 hour stage, the whole of the abdomen becomes externally segmented into eleven segments and thus the definitive body segmentation is established.

It should be pointed out that in the 59 hour stage (fig. 13) the embryo passes through an extremely long and thin stage, when it measures about 2·5 mm in length and about 0·23 mm across the metathorax. Subsequently, it undergoes an actual

shortening in length, and becomes stouter as already seen in the 60 hour stage (fig. 14), when it measures about 2·3 mm in length and 0·35 mm across the metathorax. After this, the absolute length of the embryo increases but the length in proportion to its width decreases, so that the embryo looks short and stumpy. After blastokinesis the ratio of length to width again increases. Shortly before the beginning of blastokinesis the eye-pigment first makes its appearance as a faint orange-coloured area at the posterior border of the large rudiments of the compound

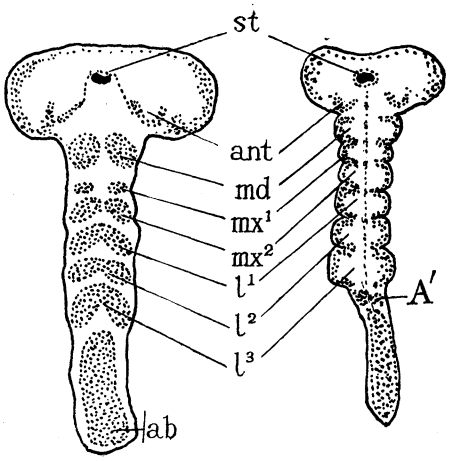

Fig. 11.

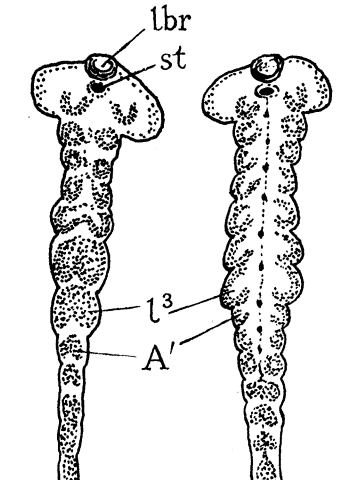

Fig. 12.

Fig. 13. Fig. 14.

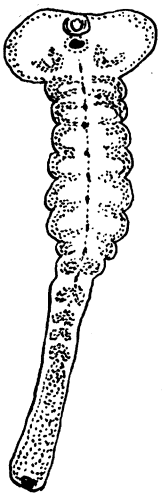

Fig. 15.

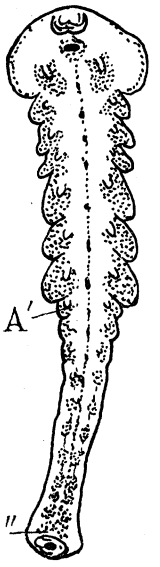

Fig. 16.

Figs. 11–16—Early germ bands. A′, first abdominal appendage ; *ab.*, abdomen ; *ab″.*, eleventh abdominal segment ; *ant.*, antenna ; *l.*¹–*l.*³, first to third thoracic legs ; *lbr.*, labrum ; *md.*, mandible ; *mx*¹., first maxilla ; *mx*²., labium ; *pr.*, proctodaeum ; *st.*, stomodaeum.

Fig. 11—Germ band 52 hours old. The rudiments of the jaw appendages and the thoracic legs are seen. × 45.

Fig. 12—Germ band 53 hours old. Definitive external segmentation of the body has begun. The rudiments of the first abdominal appendages are seen. × 30.

Fig. 13—Germ band 59 hours old. It is very long and thin. × 30.

Fig. 14—Germ band 60 hours old. It is becoming shorter and stouter. × 30.

Fig. 15—Germ band 65½ hours old. × 30.

Fig. 16—Germ band 70 hours old. The eleven abdominal segments are clearly seen. × 30.

eyes. As pointed out by Slifer (1932, *a*), after blastokinesis " the state of development of the hind femora is perhaps the best criterion to be used in estimating the age of such embryos ".

It will be seen from the above account that the definitive body segmentation of the locust embryo is preceded by a transient primary segmentation into four " macromeres " or " macrosomites ", exactly like what obtains in *Donacia* (Hirschler,

1909). This primary segmentation is shared by the inner layer also. It was first observed in insect embryos by AYERS (1884) in *Oecanthus*. Afterwards, GRABER (1888, *b*), NUSBAUM (1889), KULAGNIN (1898), HIRSCHLER (1909), SEIDEL (1924) and GRANDORI (1932) have reported it in several other insects belonging to different orders. In the Acridid *Stenobothrus*, GRABER (1888, *b*) was able to observe primary segmentation in the inner layer only, and not in the external body form. NELSEN (1931, 1934, *a*) and SLIFER (1932, *a*) make no mention of the primary segmentation in *Melanoplus differentialis*.

It is with great pleasure that I take this opportunity of expressing my sincere thanks to Professor J. STANLEY GARDINER, F.R.S., for accommodating me in the Zoological Laboratory, Cambridge. To Dr. A. D. IMMS, F.R.S., I owe a deep debt of gratitude for his kindly guidance and friendly counsel throughout the progress of this work. Part of this work was done in the Kaiser Wilhelm-Institut für Biologie, Berlin-Dahlem, during the summer of 1934. To Professor RICHARD GOLDSCHMIDT I am grateful for giving me a table in the Institute. I am indebted to the Alexander von Humboldt-Stiftung, Berlin, for a grant to enable me to continue this work in Berlin. To Fräulein Dr. ELISABETH HÖNER and to Dr. H. W. LISSMANN I wish to express my thanks for their help in the preparation of some of the illustrations. Finally, I would like to thank Professor L. E. S. EASTHAM for suggesting some improvements in the text.

IV—APPENDIX.

While this work was in the press there has appeared an important paper by the late A. J. THOMAS (1936) on the embryonic development of *Carausius morosus* (Phasmidae). The following remarks are made on this paper, so far as it touches the present work.

Formation of the Inner Layer.—The inner layer of *Carausius* arises, according to THOMAS, by the method of invagination, *i.e.*, by proliferation from the roof of a mid-ventral groove, called by him the " gastral furrow ". It is interesting to note that previous workers on the same insect (HAMMERSCHMIDT, 1910 ; STRINDBERG, 1914; and LEUZINGER, 1925) describe the inner layer as arising by immigration, without the formation of a mid-ventral groove.

A point of extreme interest is the discovery by THOMAS in *Carausius* of an " anterior ventral groove " which precedes, in time, the " gastral furrow ". The latter is formed after, and as a continuation of, the former. The " anterior ventral groove " of *Carausius* recalls a similar structure described by EASTHAM (1927) in *Pieris*, with this difference, that whereas in *Pieris* the cells budded off from the roof of this groove degenerate, in *Carausius* they form the yolk-cell membrane (*vide* below). Needless to say, the two grooves in *Carausius* correspond to the " first " and " second " ventral

grooves of *Locusta* and furnish an additional support to the idea of multi-phased gastrulation among insects. It forcefully confirms my previous conclusions that more exact work on the early stages of insect embryology will bring to light the existence of structures such as supernumerary ventral grooves, etc., which may represent some phase of gastrulation.

Yolk-Cell Membrane.—THOMAS confirms the presence of the yolk-cell membrane in *Carausius morosus* described by the previous writers cited above. He also agrees with them in regarding it as the primary endoderm which " might represent the vestiges of an ancient mid-gut epithelium which was primitively formed from the yolk cells ". But his statement (p. 502) that this membrane was regarded by HAMMERSCHMIDT (1910) and LEUZINGER (1925) to give rise to the lining of the mid-gut, is inaccurate. LEUZINGER and WIESMANN (1925) clearly state (p. 102) that it only serves as a sliding path (" Gleitbahn ") for the compensatory or replacement cells (" Ersatzzellen "=secondary endoderm) which ultimately form the mid-gut epithelium.

The most important part of THOMAS's finding in regard to the yolk cell membrane is the determination of its precise mode of origin, which was not so clear to the older authors. It arises as a proliferation of cells from the middle of the germ band, and is preceded, in point of time, by the first (" anterior ") ventral groove. Thus, it is clearly demonstrated in *Carausius* that the first ventral groove is a kind of gastrulation groove whose existence is related to the formation of the primary endoderm (yolk-cell membrane). Both these structures are formed in *Locusta* where, however, they make their appearance simultaneously. The second ventral groove of *Carausius* is related to the formation of the inner layer. The example of *Carausius* thus conforms to the classical mode of animal gastrulation in which at first the ectoderm and endoderm are differentiated and later the mesoderm, the only difference being in the formation of two gastral grooves, instead of one, in *Carausius*.

The recent paper of DRUMMOND (1936) on *Ephestia Kuhniella* may also be mentioned here. In this apparently inexact and superficial work, she claims that the inner layer arises from a middle plate which is later overgrown by the lateral plates ; no mid-ventral groove is formed. This is in contradiction to the findings of SEHL (1931) on the same insect. He shows (p. 582) the formation of a clear gastral groove (" Primitivrinne ") from the roof of which the inner layer arises. It is strange that DRUMMOND makes no reference to the important work of SEHL.

Finally, I should take this opportunity of mentioning the occurrence of certain supernumerary ventral grooves which have come to my knowledge since the paper was communicated to the Society. GRABER (1889) has shown that in *Calliphora* three parallel gastral grooves occur instead of a single one. He regards this as an instance of " lateral " gastrulation. HEYMONS (1895) has shown a similar condition to obtain in *Periplaneta*. The theory of multi-phased gastrulation propounded in this paper had mainly contemplated the elongation of gastrulation in time and its

subsequent splitting up into several phases. The two examples cited above would seem to show that the elongation of the gastrulation phase can occur not only in time but also in space. The term " multi-phased gastrulation " should, therefore, be extended to include both of these cases.

V—Summary

For the study of embryonic development, eggs were incubated at a constant temperature of 33° C, and in moist sand.

The earliest cleavage stage described is the 4-cell stage. The cleavage cells lie near the posterior pole of the egg where, presumably, fertilization takes place.

The cleavage cells divide most rapidly during the 7 to 10 hour period, after which there is a progressive slowing down. The rate of migration of cleavage cells towards the anterior pole of the egg rapidly rises after the 10 hour stage. Migration takes place along the egg-periphery.

The formation of the primary epithelium (= " blastoderm ") first takes place at the posterior end of the egg and thence travels anteriorly. Intravitelline separation occurs, thus giving rise to primary yolk cells.

At the 30 hour stage, a shallow groove termed the " first ventral groove " is formed in the mid-ventral line of the anterior portion of the germ disk. It lasts for about 4 hours or less and is not connected with the differentiation of the inner layer. At the same stage, a yolk-cell membrane is formed on the ventral side of the embryo. It disappears within four hours.

The " second ventral (gastral) groove " appears at the 42 hour stage and lasts for 3–4 hours. The inner layer is proliferated from its roof.

The structure of the yolk, secondary yolk cleavage, etc., are described. Secondary yolk cells may arise either by multipolar migration inwards or by division of primary epithelium cells, the latter being a new mode not so far recorded among insects.

A new theory of multi-phased gastrulation among insects is proposed and discussed. It regards insect gastrulation as occurring in several phases.

The homologies of the primary epithelium, primary and secondary yolk cells, ventral grooves and the inner layer are discussed.

Both the amnion and the serosa are present and arise in the usual way. Their relation to the germ band and to each other is such that there obtains a condition which is intermediate between the superficial and the submerged types of germ bands.

Changes in the external form of the embryo are described. A primary segmentation of the body (involving both the ectoderm and the inner layer) into four elements precedes the definitive one.

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VII—Explanation of Plates

All figures, except where otherwise stated, are from camera-lucida drawings. The age of the stages referred to signify the period after egg-laying when kept at a constant temperature of 33° C and on moist soil (humidity near saturation).

Lettering

a., extent of distribution of cleavage cells at the 8 hour stage ; A′.–A²., first and second abdominal appendages ; *ab.*, abdomen ; *ab″.*, eleventh abdominal segment ; *am.*, amnion ; *am.cv.*, amniotic cavity ; *ant.*, antenna ; *c.*, cells proliferating from the roof of the first ventral groove ; *c.cl.*, cell clusters ; *cdl.*, caudal end ; *cl.c.*, cleavage cells ; *cph.*, cephalic end ; *deg.y.c.*, degenerating yolk cells ; *ect.*, ectoderm ; *em.mem.*, embryonic membranes ; *em.obr.*, embryonic portion of the primary epithelium ; *e.w.*, egg-wall ; *ex.obr.*, extra-embryonic portion of the primary epithelium ; *f.*, negative images of fat globules ; *f.v.g.*, first ventral groove ; *g.b.*, germ band ; *g.d.*, germ disk ; *h.l.*, head lobes ; *in.l.*, inner layer ; l^1.–l^3., first to third thoracic legs ; *lbr.*, labrum ; *l.c.*, large cell ; *m.c.*, micropylar canal ; *md.*, mandible ; *micr.*, probable micro-organisms ; mx^1., first maxilla ; mx^2., second maxilla or labium ; *nu.*, nucleus ; *obr.*, primary epithelium ; *pcl.*, protocephalon ; *pcr.*, protocorm ; *per.*, periplasm ; *pr.*, protoplasm ; *p.y.c.*, primary yolk cells ; *s.*, swellings on either side of the second ventral groove ; *ser.*, serosa ; *st.*, stomodaeum ; *s.v.g.*, second ventral groove ; *s.y.c.*, secondary yolk cells ; *vac.*, vacuole ; *y.*, yolk ; *y.c.m.*, yolk-cell membrane ; *y.c.s.*, yolk-cell syncytia ; *z.*, yolk particles probably in a phase of digestion ; I, II, III, IV, primary segments of germ band.

Plate 33.

Fig. 17—Portion of a longitudinal section near the posterior pole of a 13 hours-old egg. The cleavage cells are near the egg-periphery. × 370.

Fig. 18—The same of an 18 hours-old egg. The cleavage cells have reached the egg-periphery where they flatten out temporarily. Some yolk particles, probably in a phase of digestion, can be seen at *z*. × 370.

Fig. 19—Transverse section near the posterior pole of an 18 hours-old egg. × about 37.

Fig. 20—Portion of a longitudinal section near the posterior pole of a 21 hours-old egg, showing the tendency of the cleavage cells to form groups. The cells have lost their flattened form (*cf.* fig. 18) and are again rounded or oval. × 370.

Fig. 21—The same from a 23 hours-old egg. The cells have formed a continuous layer on the egg-periphery at the posterior end of the egg. A secondary yolk cell is seen migrating inwards from the periphery (*cf.* fig. 24). × 370.

Fig. 22—Transverse section near the posterior pole of an egg about 23 hours old, showing the primary epithelium on the ventral side. × about 37.

Fig. 23—Portion of a longitudinal section of an egg of the same stage as in fig. 20, showing a cell near the anterior end of the egg. Compare this cell with those in fig. 20. × 370.

Fig. 24—Portion of a longitudinal section near the posterior pole of an egg about 23 hours old, showing two stages in the formation of secondary yolk cells by migration (*cf.* fig. 21). × 370.

Fig. 25—Transverse section near the posterior end of an egg 28 hours old. × about 100.

Fig. 26, *a*, *b*—(*a*). Part of the ventral (embryonic) region in fig. 25 more magnified. Note the flattening of yolk cells close to the germ disk. They are on their way to form the yolk-cell membrane. × 370. (*b*). The same, but showing the region where the embryonic and extra-embryonic regions of the primary epithelium meet. × 370.

Fig. 27—Portion of a transverse section near the posterior end of an egg 30 hours old, showing the first ventral groove, the yolk-cell membrane and the multi-layered condition of the germ band. × about 167.

Fig. 28—Portion of the same more magnified, showing the first ventral groove and the rounded cells proliferating from its roof. × 370.

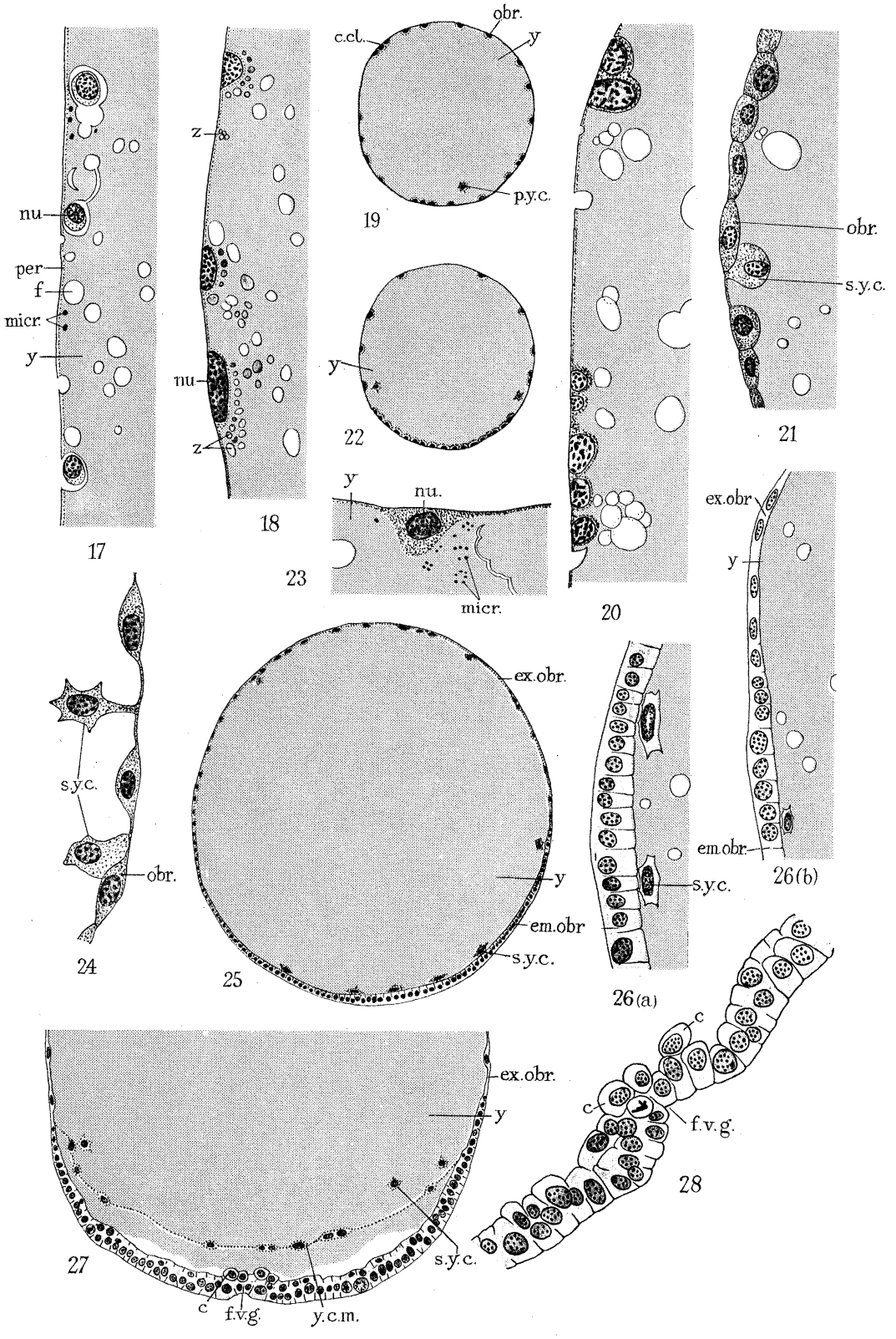

Plate 34.

Fig. 29—Portion of a longitudinal section of the posterior end of an egg 30 hours old, showing the first ventral groove and the yolk cell membrane. × about 92.

Fig. 30—Portion of a longitudinal section of an egg 40 hours old, showing the extra-embryonic region of the primary epithelium and the yolk-cell syncytia. × 370.

Fig. 31—Portion of a transverse section of the posterior end of an egg about 34 hours old, showing the germ band region. Note the absence of a single-layered condition in the germ band. A very large cell is seen on the left; it is not a genital cell. Some of the degenerating yolk cells are seen. × 210.

Fig. 32—Transverse section of the posterior end of an egg about 42 hours old, passing across the hind region of the protocephalon, and showing the embryonic membranes and the inner layer. × about 92.

Fig. 33—Portion of a transverse section across the middle region of a 45 hours-old germ band, showing the second ventral groove and the differentiation of the inner layer. × 520.

Fig. 34—Portion of the same as in fig. 32 more magnified. × 230.

Fig. 35—Portion of a transverse section of the posterior end of an egg about 45¾ hours old, passing through the middle of the germ band. Membrane formation is not yet complete in this region. × 155.

Fig. 36—Transverse section across the posterior region of the protocorm of a germ band about 46 hours old, showing the thick inner layer. × 170.

Fig. 37—Serosa at the posterior pole of an egg about 40 hours old. The cells at the pole form a circular area of comparatively small and closely packed nuclei. × 95.

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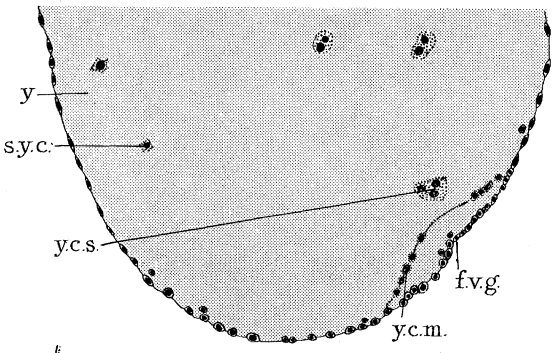

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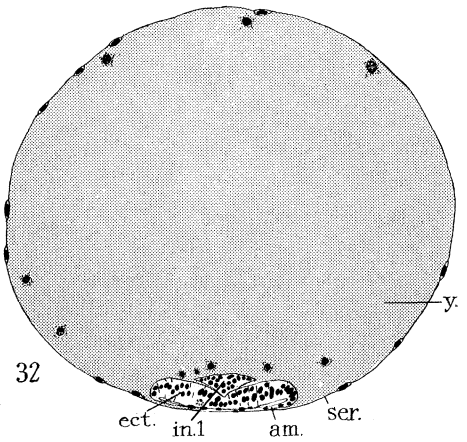

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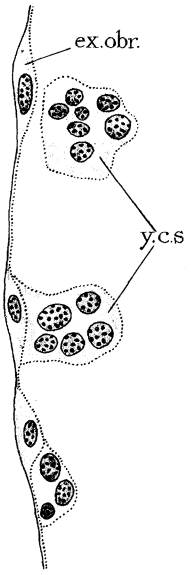

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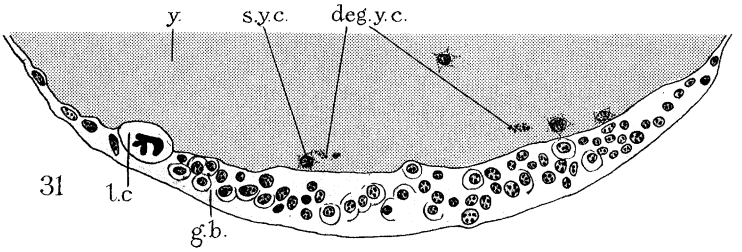

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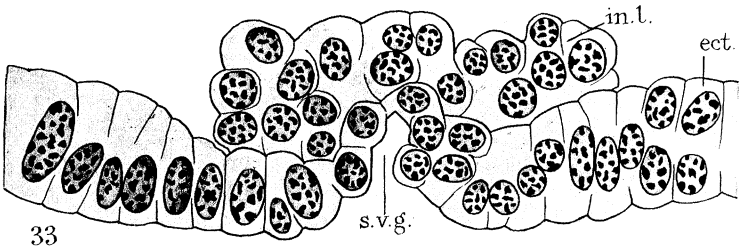

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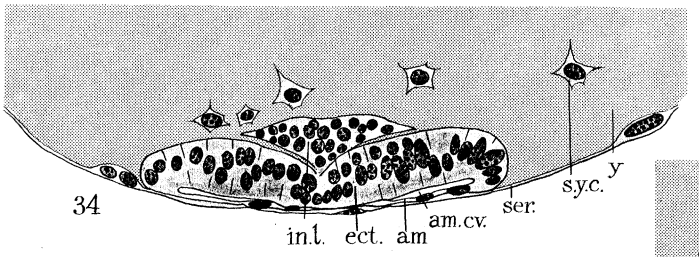

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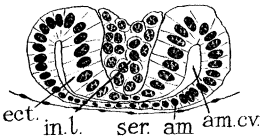

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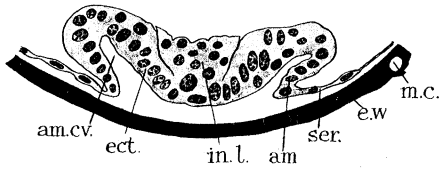

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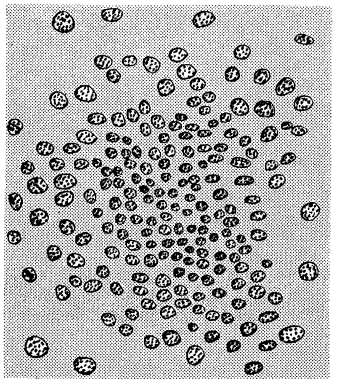

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Plate 35.

Fig. 38—Longitudinal section of the posterior end of an egg 46 hours old, passing medianally across the germ band. × 170.

Fig. 39—Surface view of the posterior end of an egg 21 hours old, after removal of the egg-wall, showing groupings of cleavage cells prior to the formation of the germ disk. × 56.

Fig. 40—The same from a 22 hours-old egg, showing the germ band distinctly marked out and its component cells arranged more or less regularly. × 56.

Fig. 41—The same from an egg 28 hours old. × 56.

Fig. 42—Germ band 42 hours old. The embryonic membranes have been formed at the cephalic end, but the caudal end is still free from them. The second ventral groove is beginning to form at the cephalic end. × 56.

Fig. 43—Germ band about 46 hours old. The embryonic membranes are seen at both the cephalic and the caudal ends, but the middle region of the germ band is still free from them. The area of inner layer differentiation has travelled backwards and reached the caudal extremity. The apparent bifurcation of the inner layer in the cephalic region represents the swellings of the germ band on either side of the second ventral groove. Note also the two lateral bulges in the middle of the protocorm. × 56.

Fig. 44—Germ band about 50 hours old. The formation of the embryonic membranes is complete. The inner layer is beginning to segment. Note the stomodaeal rudiments and the specially well-developed head lobes. × 56.

Fig. 45—The same but a slightly older stage. The germ band has divided into four primary segments (I–IV) and the segmentation of the inner layer roughly corresponds to this. The antennary rudiments are seen. × 56.

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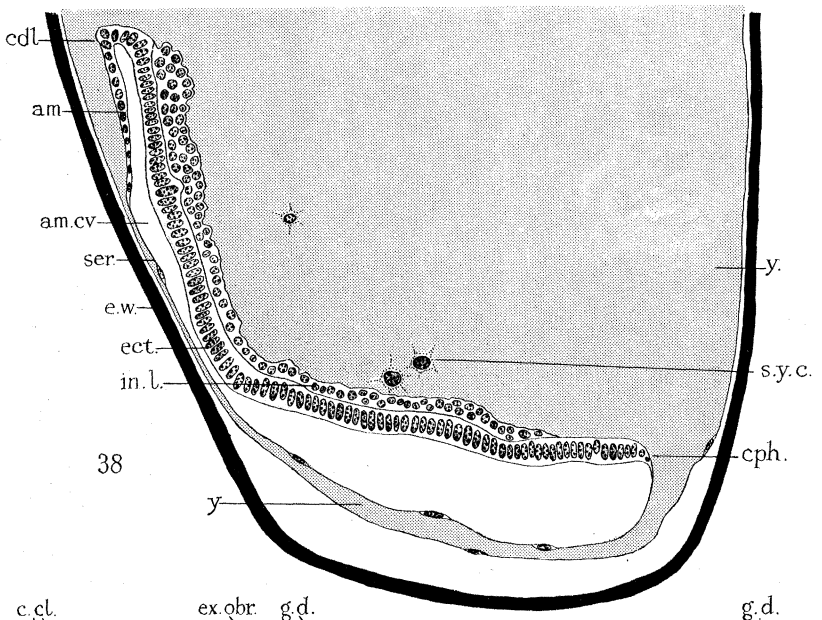

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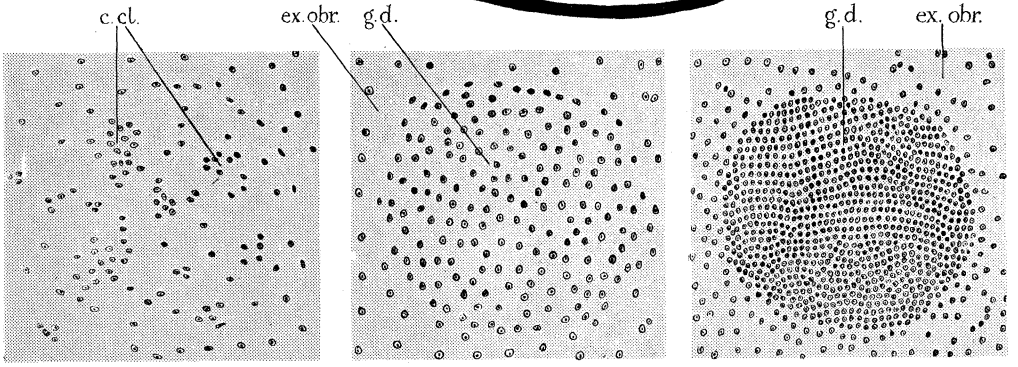

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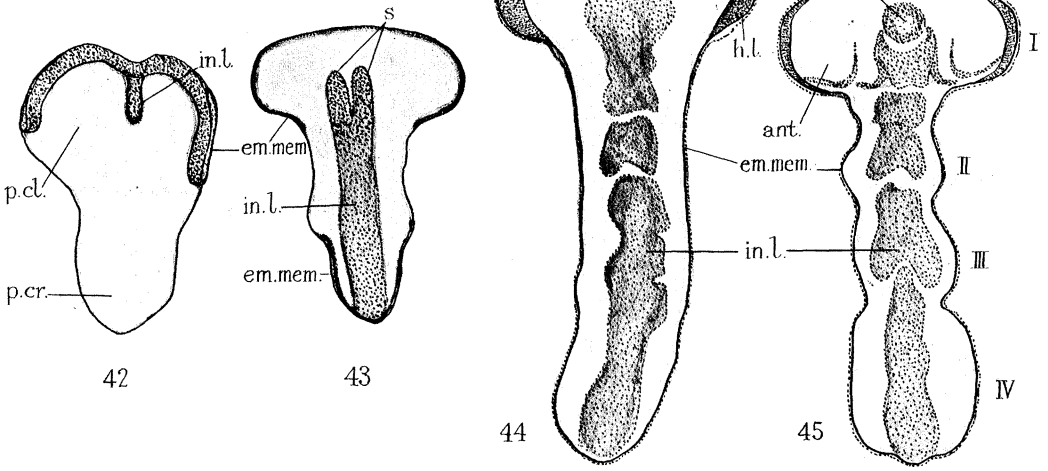

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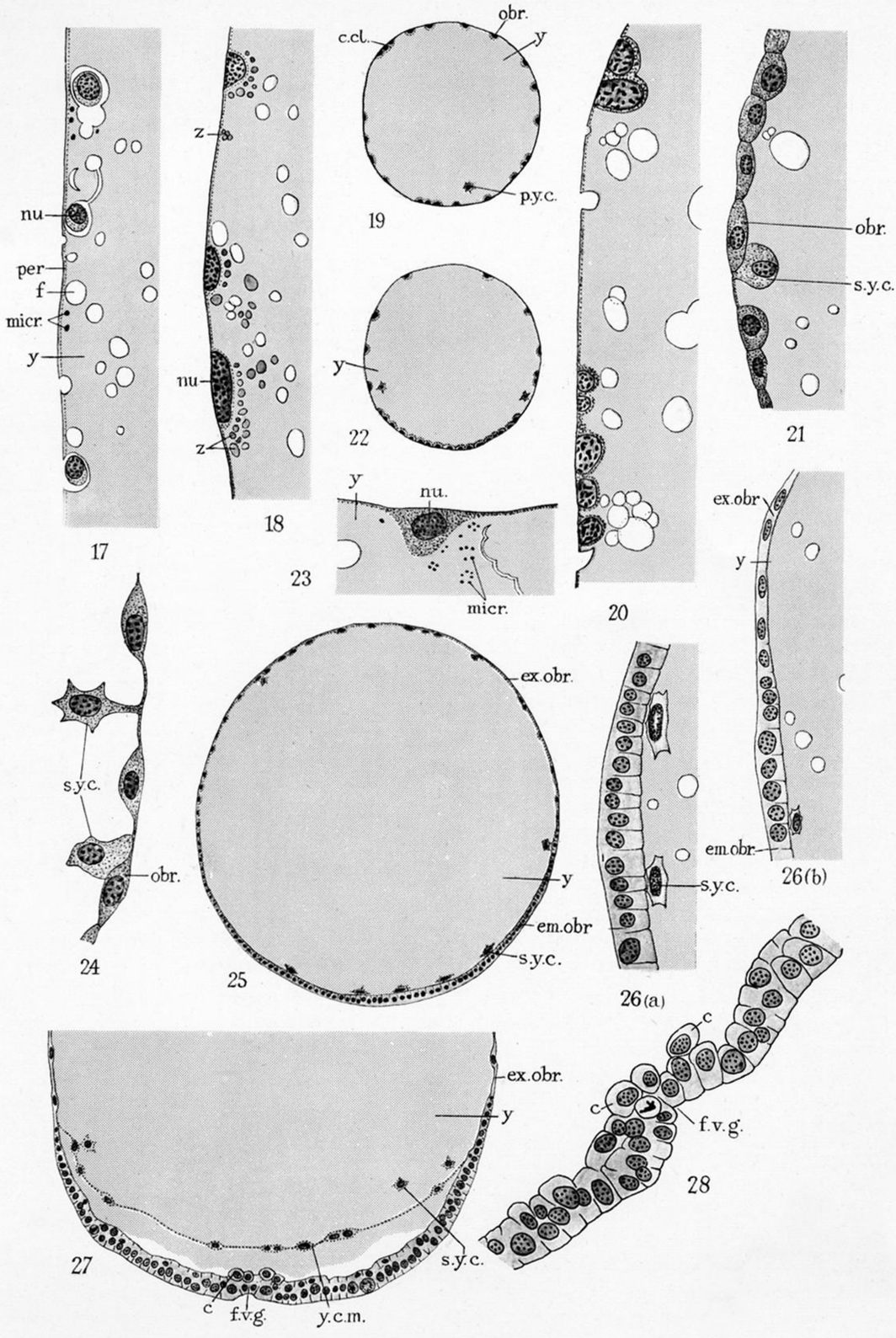

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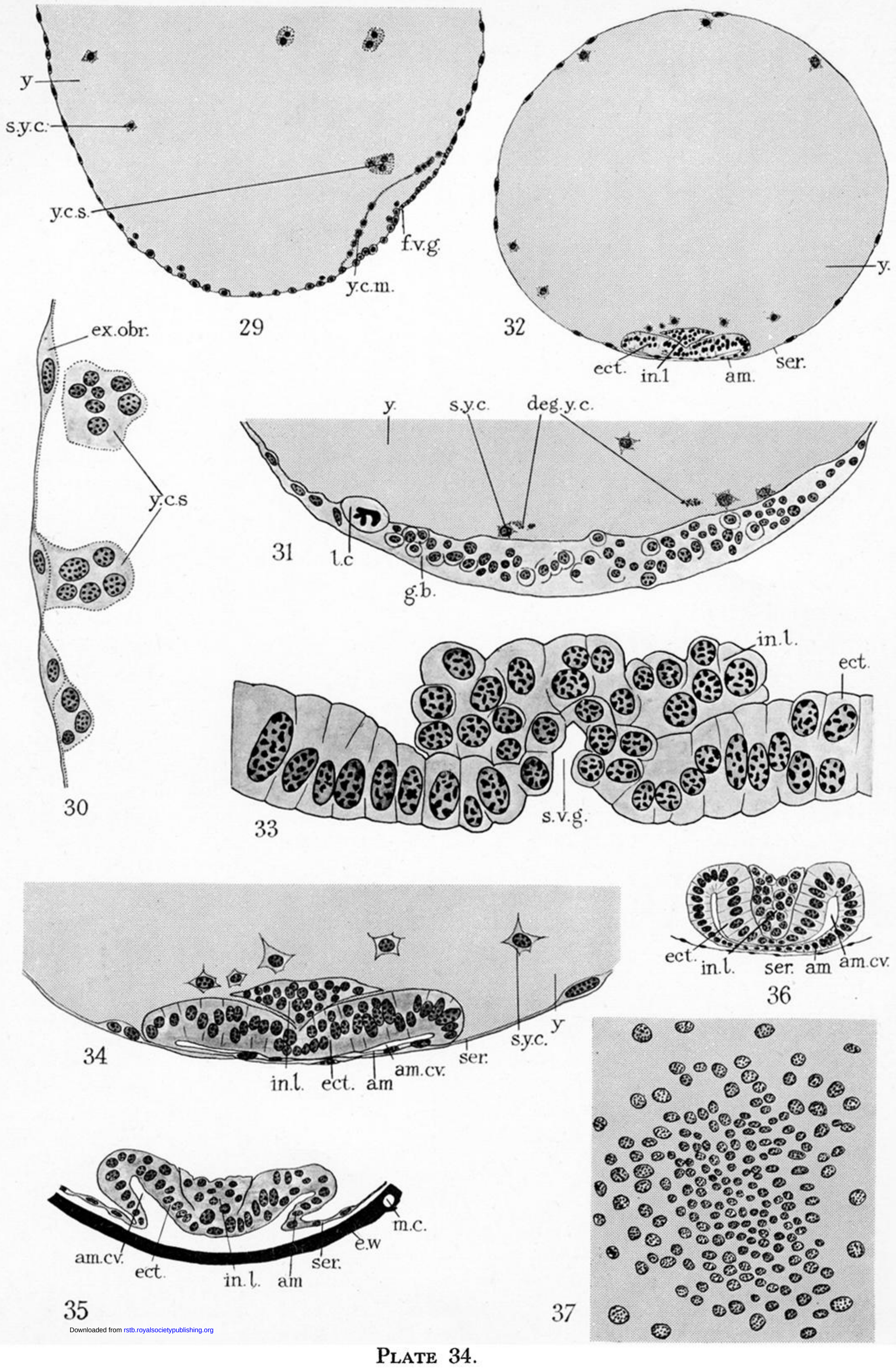

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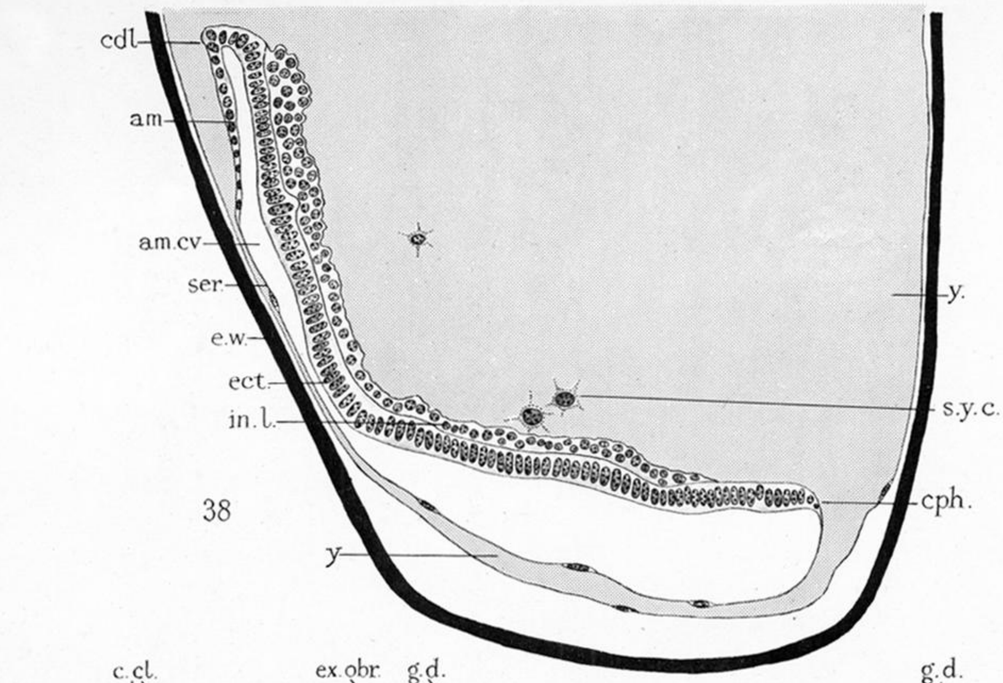

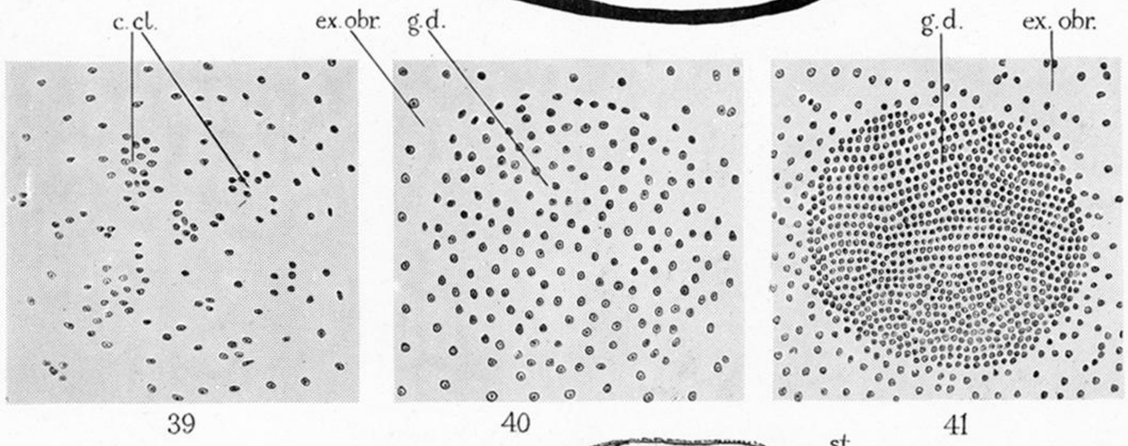

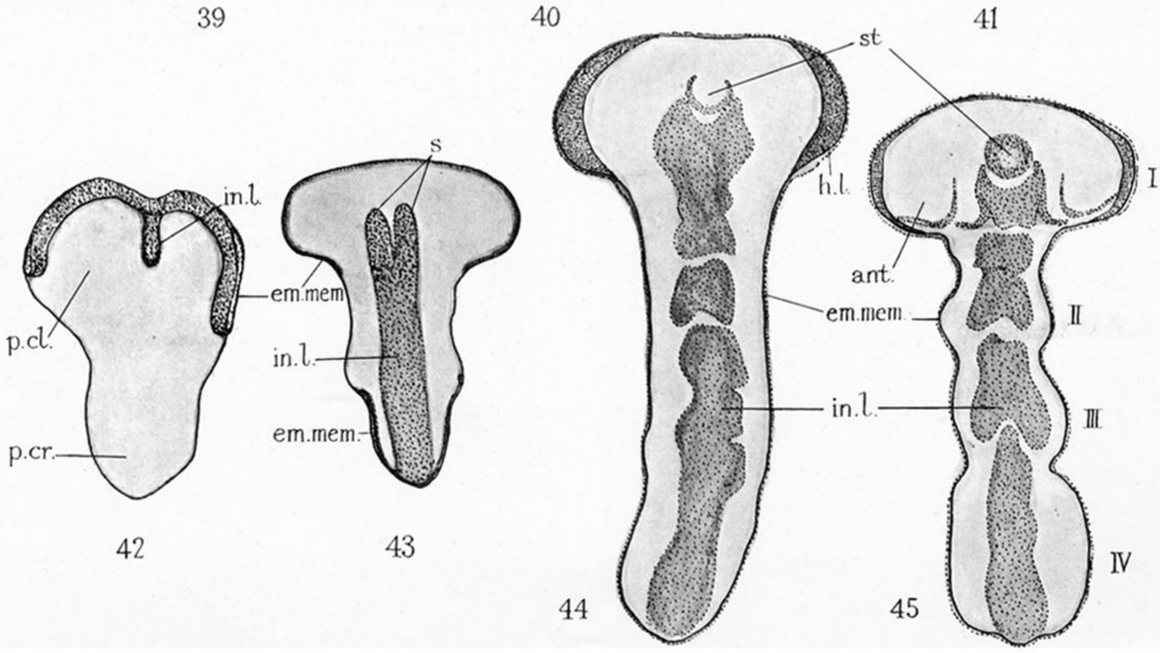

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