The Embryonic Development of Calandra oryzae.

By

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With Plates 21–26 and 19 Text-figures.

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1 Much of the section-cutting and drawing has been the work of Miss Murray, whom I therefore wish to include as author of this paper. O.W.T.
The following observations on the embryology of the rice weevil were intended, in the first place, as a contribution to the discussion on the formation of germ-layers in the insect embryo. An account of the post-embryonic development of this species having already been given (Murray and Tiegs, 1935), the observations were extended to cover the whole embryonic period; accordingly a reasonably ‘complete’ description of the development of this insect from egg to imago is now available.

Taken as evidence bearing on the phylogeny of the Insecta, the embryology of a specialized form like Calandra is necessarily inferior in value to that of the more archaic types; but for the purely formal problems of insect development,
which alone have been considered here, it is the ready supply of technically suitable material that must decide the choice of subject, and as such Calandra has proved very satisfactory.

The extensive literature on insect embryology is remarkable for the diversity of opinion expressed even on matters of direct observation, important theoretical questions, notably a possible conflict with the germ-layer theory, being involved. It is therefore desirable that an intensive study be made of some readily procurable cosmopolitan species, rather than of numerous diverse forms which render comparison difficult. In the present case three relevant investigations are available—those of Tichomirow (1890) and of Inkmann (1933) on Calandra granaria, and Mansour's work on Calandra oryzae (1927).

Tichomirow's work is in the form of only a very brief note. Mansour's paper is concerned mainly with mid-gut development, but contains incidentally other observations of importance. Inkmann's work is devoted chiefly to the early phases of development, but mid-gut formation is also described.

The controversy over mid-gut development is well exemplified in these three papers, Tichomirow deriving it from the yolk-cells, Mansour from the proctodaeum and stomodaeum, while Inkmann alone supports the orthodox view of an endodermal origin.

MATERIAL AND METHODS

The weevils may readily be bred in captivity from wheat grains. The female lays her eggs singly in holes excavated with the rostrum in the grain, the hole being then sealed with a gluey secretion extruded from the ovipositor. The presence of this plug, easily recognized when the surface of the wheat grain is scanned under a low-power binocular lens, affords an easy means of detecting the egg. Infected grain is kept in an incubator at 25-6° C.

The egg is extracted by removing the hard shell of the wheat and breaking away as much of the endosperm as possible, without injury to the egg. Thus partially exposed it is immersed in saline, which softens the remaining endosperm, and enables the egg to be freed. Softening of the grain, by soaking in water
previous to exposure to the weevils, facilitates removal of the egg. Even with the utmost care, however, eggs, particularly if recently laid, are often broken during removal, for they are very fragile.

Of the various standard fixatives, those of Bouin, Gilson, Carl, and Bles give excellent results, particularly when used hot; with Heidenhain’s ‘Susa’ mixture, which Inkmann recommends, the results were not as good, while Carnoy’s fixative proved inferior. Mostly Carl’s fixative has been employed, the eggs being immersed for 15 minutes in the fixative at 60°C. The fixation is usually very good, though occasionally the fixative fails to penetrate the chorion, while in some cases the egg bursts. The eggs are then transferred for a day to 70 per cent. alcohol, where the fragile chorion is partly or wholly removed with fine needles.

For staining whole embryos, by far the most satisfactory preparations have been made by use of the Feulgen method as applied recently by Schmuck and Metz (1931) and by Du Bois (1982) to whole eggs and embryos. With this method the chromatin is selectively stained, the embryo thus becoming sharply defined; the yolk, which remains uncoloured, may be counterstained with ‘light green’. The method requires some practice, for overstaining easily occurs. Useful preparations can also be obtained with Auerbach’s methyl green—acid fuchsin mixture. The embryos are cleared in clove oil and mounted in thin balsam, so that they can easily be rolled over under the cover-glass and examined from all angles.

For sections the celloidin-paraffin double-embedding method has been used, the ordinary paraffin procedure proving unsuitable for embryos. For the study of maturation and fertilization, however, paraffin embedding is quite adequate, for the yolk is not unduly hardened by xylol.

RATE OF DEVELOPMENT

To facilitate description the successive stages of development will be given in terms of the age of the embryo. It is not, of course, inferred that the rate of development is even approximately constant at fixed temperature; nor even that the relative
rate of development of different organs is the same for various embryos. In all cases the age assigned to a particular stage of development is the minimum age at which it has been found to appear.

The following table has been constructed for a temperature of 26° C.:

<table>
<thead>
<tr>
<th>Age of Embryo</th>
<th>Stage of Development</th>
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<tbody>
<tr>
<td>Newly laid</td>
<td>Equatorial plate of first meiotic division (fig. 4, Pl. 21).</td>
</tr>
<tr>
<td>10 min.</td>
<td>First meiotic anaphase (fig. 5, Pl. 21).</td>
</tr>
<tr>
<td>25 min.</td>
<td>First polar body (fig. 9, Pl. 21).</td>
</tr>
<tr>
<td>65 min.</td>
<td>Second meiotic anaphase (fig. 14, Pl. 21).</td>
</tr>
<tr>
<td>80 min.</td>
<td>Just prior to fertilization (fig. 17, Pl. 21).</td>
</tr>
<tr>
<td>110 min.</td>
<td>7 cleavage-cells.</td>
</tr>
<tr>
<td>7 hr.</td>
<td>Peripheral distribution of cleavage-cells (Text-fig. 2 a).</td>
</tr>
<tr>
<td>7½ hr.</td>
<td>Cleavage-cells entering periplasm (Text-fig. 3 a).</td>
</tr>
<tr>
<td>12 hr.</td>
<td>Blastoderm; partitions between adjacent cells present but internal cell-wall unformed; secondary periplasm forming (fig. 30, Pl. 22).</td>
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<tr>
<td>17 hr.</td>
<td>Blastoderm as in Text-fig. 3 b.</td>
</tr>
<tr>
<td>20 hr.</td>
<td>Blastoderm; internal cell-walls fully developed; germ-cells invaginating (Text-fig. 3 d).</td>
</tr>
<tr>
<td>22-3 hr.</td>
<td>Lateral and median plates differentiating from blastoderm; embryonic membranes appearing (Text-fig. 4).</td>
</tr>
<tr>
<td>25 hr.</td>
<td>Dorsal flexure developing (Text-fig. 5).</td>
</tr>
<tr>
<td>27 hr.</td>
<td>Dorsal flexure further developed; head-lobes present; embryonic membranes do not yet enclose amniotic cavity (Text-fig. 7).</td>
</tr>
<tr>
<td>28 hr.</td>
<td>Appearance of mandibular segment (Text-fig. 8).</td>
</tr>
<tr>
<td>30 hr.</td>
<td>Maximum development of dorsal flexure; gnathal appendages appearing; embryonic membranes now enclose amniotic cavity; thorax segmenting; stomodaeal component of mid-gut arising.</td>
</tr>
<tr>
<td>38 hr.</td>
<td>Gnathal appendages prominent; segmentation extending along abdomen (Text-fig. 9).</td>
</tr>
<tr>
<td>42 hr.</td>
<td>Thoracic appendages present; abdomen segmented (Text-fig. 10).</td>
</tr>
<tr>
<td>48 hr.</td>
<td>Thoracic appendages prominent; somites with well-developed coelomic sacs; malpighian tubes and tracheal system appearing; proctodaeal component of mid-gut not developing yet.</td>
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<th>Age of Embryo</th>
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<tr>
<td>60 hr.</td>
<td>Shortening of germ-band beginning (Text-fig. 12); procto-deal component of mid-gut present.</td>
</tr>
<tr>
<td>70 hr.</td>
<td>Shortening advanced (Text-fig. 13). Anterior and posterior components of mid-gut have met.</td>
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<tr>
<td>75 hr.</td>
<td>Shortening complete.</td>
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<tr>
<td>96 hr.</td>
<td>Larva emerges.</td>
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OBSERVATIONS

1. MATURATION AND FERTILIZATION OF THE EGG

A. Structure of the Egg.—The egg is semi-transparent, ovoidal, and rather more pointed at its anterior than posterior end; it measures on the average 0·6 mm. long, 0·27 mm. broad.

The chorion is uncoloured and unsculptured, and is very fragile.

Directly investing the egg protoplasm is a vitelline membrane, considerably thinner even than the chorion.

The egg-cytoplasm is concentrated mainly round the periphery of the egg as the periplasm (Keimhautblastem of Weismann), and two zones are usually distinguishable in it, an outer thinner and more eosinophil, and an inner granulated and more basophil layer. This stratification of the periplasm is recognizable in various figures on Plate 21, and recalls that already described for certain Lepidoptera (Schwangart, Huie, Eastham, Johannsen). The inner surface of the periplasm has a ragged appearance owing to the presence of the fine branching strands of protoplasm which spread inwards to form an anastomosing mesh supporting the entire yolk (fig. 23, Pl. 22; also various figures on Plate 21). The periplasm is of fairly uniform thickness except at the anterior pole of the egg, where it increases in quantity.

The yolk-grains are of variable size; occasional clear spaces between them suggest the presence of scattered oil-vacuoles.

The nucleus of the freshly laid egg lies within the periplasm, and is in the prophase of the first meiotic division. The diploid chromosome number is twelve (fig. 22, Pl. 21).

The orientation of the egg follows the usual rule, the morpho-
logically anterior end lying also anterior in the ovarian tube. There is no means of distinguishing dorsal from ventral surface.

B. Maturation.—The maturation phenomena that occur after laying are difficult to interpret without an examination of the unlaid egg. At first sight they seem to show post-reduction, and were described thus by Inkmann (1933) for Calandra granaria. Actually an obscured form of pre-reduction occurs.

An egg from the lower end of the ovarian tubes presents the following features (Text-fig. 1). The cytoplasm is already mainly concentrated at the periphery as the periplasm, from which arises the fine meshwork of anastomosing filaments that support the yolk in the interior of the egg. At the anterior pole the periplasm, at this stage, projects like a large plug into the yolk. Within the yolk lies a prominent spherical body with eosinophil granular protoplasm, and with a well-defined investing membrane (Text-fig. 1, pnb.). It is apt to be taken for the nucleus. It does not, however, give the specific Feulgen reaction; and moreover, as its development and subsequent history show, it is a specialized mass of cytoplasm with the true nucleus, invested by a peculiarly wrinkled sheath (figs. 1 and 2, Pl. 21), lying in its interior. In the oogonia, which form the tip of the ovarian tube, this perinuclear substance does not occur, and it is only with the gradual enlargement of the oocyte that it becomes apparent as a clear zone round the nucleus, becoming very prominent farther down the tubes as the oocytes increase in size. Finally while still in the ovarian tube the body disappears, this disappearance being initiated by a rupture of the membrane, adjacent yolk-grains then invading its substance.
till eventually it ceases to be recognizable. In the meantime
the nucleus is moving towards the periphery of the egg, the
wrinkled investing sheath gradually disappearing. By the time
the egg has entered the vagina and is ready for laying the
nucleus has passed into the periplasm (fig. 3, Pl. 21).

The early maturation phenomena occur during the passage
of the oocytes along the ovarian tubes; it is not our purpose to
describe these, since in regard to these particular stages the
material is cytologically too unfavourable for such descrip-
tion to have any value. We begin the following account with
the diplotene stage of the first meiotic division (from an egg in
the last chamber of an ovarian tube). The appearance of the
nucleus at this stage is shown in fig. 1, Pl. 21; the purpose of the
illustration is to show the long filamentous form of the chromo-
somes at this period. The nucleus itself is encased in the
shrunken sheath, external to which lies the perinuclear sub-
stance, of which a small quantity only is shown.

The chromosomes now begin to shorten, while at the same
time the bivalent pairs again separate into their univalent
constituents. This condition is shown in fig. 2, Pl. 21, from an
oocyte still in the last ovarian chamber; the exconjugant

1 This body, as Text-fig. 1 shows, is so readily taken for the nucleus,
that further comments are desirable. It may be suspected that the body
is a nucleus in the germinal vesicle stage, with diffuse chromosomes, the
central body being a chromatin-nucleolus. But this is not the case, for as
fig. 1, Pl. 21, shows, the chromosomes themselves are confined within the
central body, and undergo conjugation there. A chromatin-nucleolus
stage, intervening between conjugation and polar body formation does
not, indeed, seem to occur in this species.

A review of the literature bearing on this point is beyond the scope of
the present paper. That the condition is not peculiar to Calandra may
be inferred from Wheeler's description (1889) for Blatta and Dory-
phora, in which a similar body, regarded however as a nucleus, was
observed to fragment shortly before maturation, in a manner recalling
the above account for Calandra; while Henking (1887), using a defective
technique in vogue at the time, concluded that in a species of phalangid
investigated by him the nucleus even completely disappeared before polar
body formation; cf. also Ayres' (1884) account for Oecanthus.

Possibly the body is related to the 'pallial substance' that has been
described as investing the nucleus in the eggs of certain arthropods. (See
Wilson's 'Cell', 1925, p. 341.)
chromosomes have contracted into very short thick bodies, 11 of the 12 being visible in the section, most of these, moreover, appearing almost split into two. Fig. 8, Pl. 21, shows the condition of the chromosomes from an egg removed from the vagina; the nucleus has entered the periplasm, the investing sheath has disappeared, and 12 chromosomes, some partially split into two, are distinguishable. The splitting is in preparation for a precocious separation that will occur in the first meiotic anaphase.

In the newly laid egg the chromosomes have entered the equatorial-plate stage of the first meiotic division (to obtain this stage the egg must be extracted from the wheat grain as quickly as possible after laying). The twelve univalent chromosomes have again congregated into six pairs; each chromosome is now rather more rod-shaped than in the previously described stage, the splitting not being always recognizable. For those chromosomes where the splitting is visible there is a definite indication of tetrad formation (fig. 4, Pl. 21).

About 10 minutes after laying, the egg is in the first meiotic anaphase (fig. 5, Pl. 21), the chromosomes separating into two lots of six, in many of which the tendency to precocious splitting is recognizable. Fig. 6, Pl. 21, shows the condition at late anaphase, in which the polar body is just beginning to protrude; actual division of some of the chromosomes has now occurred, and as far as it is possible to count them, there are now nine chromosomes (chromatids) in the polar body and eight at the opposite end of the spindle, an accurate count being however difficult because it is not possible to distinguish with certainty between partially and fully divided chromosomes. In the example shown in fig. 7, Pl. 21, there are nine chromatids in the polar body, nine or ten at the oocyte end, and the full diploid number is apparently in process of formation, since a few chromosomes at either pole of the spindle are evidently in a state of incomplete division.

To give objective evidence of the complete separation of the chromatids to yield the diploid number at the end of the first meiotic division, the photograph shown in fig. 8, Pl. 21, from a stage a little in advance of that shown in the previous figure, is offered. It is from a longitudinally cut egg, so that the polar body
is not present in this, but in the adjacent section; the chromatids by good fortune are all within the plane of focus, eleven being recognizable, with one of them as yet incompletely divided.

It appears then that by the end of the first meiotic division, although reduction has already occurred, the diploid number of chromosome elements is present, this being due to a precocious separation of chromatids late in the anaphase.

In the meantime the polar body has begun to protrude more prominently from the surface and eventually becomes almost completely constricted off; it lies at this stage in a depression on the surface of the egg (fig. 9, Pl. 21); its cytoplasm is always markedly eosinophil and the chromosomes do not become enclosed within a definite nuclear membrane.

In the oocyte nucleus, on the other hand, a nuclear membrane is produced. The twelve chromosomes, however, do not clump together but proceed forthwith to the second meiotic division. From now onwards there is a considerable accumulation of periplasm at the site of the oocyte nucleus, the latter moving away from the surface of the egg and projecting prominently into the yolk (fig. 17, Pl. 21).

The second meiotic division, which begins less than an hour after laying, first becomes recognizable by a tendency of the chromatids of the oocyte nucleus to unite again in pairs (fig. 10, Pl. 21); and the pairing may become so intimate that the two components of the pairs are hardly recognizable (fig. 11, Pl. 21). This recoupling of the chromosomes is noteworthy; it has been referred to as preceding also the first meiotic division.1

At the equatorial plate stage (fig. 12, Pl. 21) spindle-fibres have become more distinct. The anaphase is shown in fig. 13, Pl. 21; six chromosomes are moving to opposite poles of the spindle; the nuclear membrane is still intact and the spindle is

1 While reference to the extensive bibliography on maturation is beyond the scope of the present paper this peculiar phenomenon needs further comment. It was first observed by Agar (1911) in Lepidosiren, and has since been recorded by Hogben (1920) for parasitic Hymenoptera—in both cases for the first meiotic division only. The present case is therefore of unusual interest in that it precedes both meioses, the diploid number of chromosomes having been restored by precocious separation of the univalents late in the first anaphase.
completely intranuclear. Disappearance of the nuclear membrane begins in late anaphase (fig. 14, Pl. 21; 65 minutes after laying), and in the early telophase is no longer recognizable (fig. 15, Pl. 21). At this period the chromosomes have clumped together and can no longer be individually distinguished. In the late telophase the clumping is still denser. Finally, each nucleus enters the resting condition (fig. 16, Pl. 21). It is noteworthy that in this respect the nucleus of the second polar body differs from that of the first; unlike the first, moreover, it remains throughout in the periplasm, there being no protrusion of a second polar body beyond the surface of the egg.

Division of the first polar body does not occur; the precocious division of its chromosomes in the first meiotic anaphase has already been noted.

C. Degeneration of Polar Bodies.—Reabsorption of the first polar body into the periplasm begins even before the second polar body forms, several stages being seen in figs. 12, 14, 16, 20, Pl. 21. In some eggs it is completed even before fertilization (fig. 16, Pl. 21); on the other hand, we have an egg as late as the eleven-cell stage when it is still in progress.

Degeneration of the nuclei begins usually at about the time of fertilization. In the second polar body nucleus the first indication is a marked swelling; its chromatin then becomes resolved again into chromosomes, distinguishable now from those of the first body by their much greater length (fig. 20, Pl. 21). Although they sometimes appear quite normal, yet more frequently they already show a tendency to fragment and clump together, and then stain very deeply. The nuclear membrane now disappears (fig. 21, Pl. 21).

Fragmentation of the chromosomes proceeds during the early phases of cleavage. We have one egg at the eight-cell stage in which they have almost vanished, while in another at the fifty-cell stage they are still recognizable. During the process of degeneration the two lots remain for a while separate, those of the second polar body being often distinguishable, by their greater length, from those of the first (fig. 21, Pl. 21); but later as the fragmentation proceeds they mingle, become scattered as fine globules in the periplasm, and eventually disappear.
D. Fertilization (nuclear fusion).—In most of the eggs polyspermy has been observed. The sperms apparently enter at the anterior end where the periplasm is unusually thick; according to Inkmann a micropyle occurs here (Calandra granaria). They then migrate deeper into the egg and are easily detected by the prominent mass of cytoplasm within which they lie (fig. 14, Pl. 21). They are confined to the anterior third of the egg and may occur up to five in number. The sperm-head at this period is usually a very deeply staining rod; the adjacent cytoplasm is very prominent, crescentic in form, the central pale axis probably lodging the sperm-tail (fig. 23, Pl. 22).

Less than an hour after laying the sperm-heads have become converted into rounded deeply staining hyaline bodies, in which chromosomes however are not yet visible. Towards the completion of the second maturation division they have begun to assume the appearance of a resting nucleus, though still rather hyaline. By the time the female pro-nucleus has formed the male nuclei are fully developed (fig. 15, Pl. 21).

The female pro-nucleus, already at late telophase, begins to move in the direction of the male pro-nuclei. Soon, apparently, the movement becomes a very active one, for a long trail of cytoplasm is drawn out by the female pro-nucleus as it passes through the yolk (fig. 16, Pl. 21). Judging by the track of cytoplasm, the female pro-nucleus moves almost in a straight line. Unlike the male it is invested by only the smallest quantity of cytoplasm; indeed, it is sometimes reduced to an imperceptible quantity, and were it not for the trail of cytoplasm that it leaves behind, it would be difficult to locate among the yolk grains. It resembles the male pro-nucleus in appearance, but is rather larger.

The female pro-nucleus now becomes closely applied to the male, which has in the meantime enlarged; the nuclear material is again resolved into its chromosomes, which are now long delicate threads. The condensation of cytoplasm around the pairing nuclei is very prominent (figs. 17, 18, Pl. 21). The nuclei then fuse, and the chromosomes intermingle (fig. 19, Pl. 21). A resting nucleus does not seem to be reconstituted; on the contrary the zygote-nucleus appears to divide at once,
and rapidly, for of many eggs examined at this period (80–100 minutes after laying) none were obtained showing any transition between the zygote-nucleus and the two cleavage-cell stage.

The supernumerary male pro-nuclei degenerate, and indeed, surprisingly soon. We have one preparation of a partially degenerate pro-nucleus surviving the fertilization; in all other eggs they have passed beyond recognition.

2. Cleavage

In Calandra successive cleavages are, from the beginning, usually distinguished by a complete lack of synchronization; we have, for example, one egg with three cells, one only of which is dividing, another with eight cells, of which only two are in division, while in one egg with thirty-four cells only ten are in that condition. For insects this seems to be unusual, strict synchronization of mitoses being reported for various species—Hydrophilus (Heider, 1889), Donacia (Hirschler, 1909), Musca (Blochmann, 1886), Blatta and Doryphora (Wheeler, 1889), Eudemis (Huie, 1918), Pieris (Eastham, 1927), Ephestia being remarkable in that it extends to the 512-cell stage (Sehl, 1931). On the other hand, Platner (1888) finds a lack of synchronization in Liparis.

Amitosis of cleavage-cells, as reported by Wheeler for Blatta and by Strindberg for Eutermes, has not been seen.

From the beginning of cleavage the daughter-cells migrate apart, becoming gradually spread through the yolk. Here they are seen as conspicuous clumps of protoplasm, local islands in the protoplasmic mesh that pervades the whole interior of the egg, and which connects the cleavage-cells with one another, and with the periplasm, into one large syncytium. Direct connexion can, of course, only be displayed for closely adjacent cells (fig. 25, Pl. 22).

The early cleavage-cells are confined to the anterior part of the egg (Text-fig. 2 A–C). By about the 36-cell stage they have spread also into the hinder part, and show already at this period a tendency to place themselves concentrically to the periplasm (text-fig. 2 D). As cleavage proceeds this condition becomes better defined until, at about the sixth to seventh hour, the
Cleavage. Only such cells as are present in a thick median section along the egg are shown. A, 2-cell stage; B, 4-cell stage; C, 6-cell stage; D, 36-cell stage; E, about 150-cell stage. Segregation of yolk cells and blastoderm-cells apparent in D and E. Note 'comet-cells' in E.

How is this migration brought about? It is generally attributed to some form of amoeboid movement on the part of the cleavage cells. Eastham (1927) suggests, however, from his
observations on Pieris, that a centrifugal streaming of cytoplasm occurs, and that this plays a prominent part in drawing the cells to the periphery. In support of this is the fact that the cytoplasmic mesh inside the line of advancing cleavage-cells is much less conspicuous than outside that line. This has been noted also for other insects—Lasiocampa (Schwartze, 1899), Chalicodoma and Anthophora (Carrière and Bürger, 1897), Apis (Nelson, 1915), but is usually interpreted as an incorporation of the cytoplasm into the enlarging mass of cleavage-cells. Sehl (1931), however, has observed a definite streaming of the internal cytoplasm towards the ventral periplasm shortly after the beginning of cleavage (Ephestia). But for Calandra this explanation fails. A centrifugal streaming does, indeed, occur later in immediate connexion with blastoderm formation (q.v.), but prior to this there is no evidence for diminution of the internal cytoplasm. The frequent occurrence of the peculiar well-known ‘comet-cells’ seems, on the contrary, to show that the power of movement resides within the cells themselves. For example, fig. 24, Pl. 22, shows the separation that is effected at the end of the first cleavage; the advancing end blunt, with long following trail of cytoplasm, evidently suggests a cell forcing its way through the yolk.

Do the cleavage-cells spread at random through the yolk, or does the cleavage proceed according to some regular pattern as in other animals? It seems impossible to obtain microscopical evidence on this point, the spindles of dividing cells showing no recognizable orientation in respect to the egg as a whole. It should be observed that in the collembolan Tomocerus the yolk itself undergoes cleavage (Uzel, 1898), movement of cleavage-nuclei being therefore definitely circumscribed. On this point the genetic evidence seems decisive: the theory of Morgan and Bridges on the origin of gynandromorphs in Drosophila implying an absence of indiscriminate intermingling of the cleavage-cells, while the preponderance of bilateral gynandromorphs in that insect shows that the plane of initial cleavage is usually along the axis of bilateral symmetry of the imago. In the few eggs that we have at the two-cells stage in Calandra the cleavage-cells have undoubtedly
passed into opposite halves of the egg (Text-fig. 2 A; fig. 24, Pl. 22), but beyond this no definite cleavage pattern is microscopically recognizable.

3. FORMATION OF BLASTODERM AND YOLK-CELLS

A. The Blastoderm.—This forms, at the earliest, seven to eight hours after laying, 100-150 cleavage-cells being present at the time, comprising a peripheral zone of future blastoderm cells, numbering about 90 to 130, with between 15 to 30 yolk-cells scattered in the interior (Text-fig. 2 E).

Entrance of the cleavage-cells into the periplasm is not, as with most insects, confined to a particular region, but occurs uniformly over the whole surface (text-fig. 3 A).

As observed also for other insects—Neophylax (Patten), Chalicodoma and Anthophora (Carrière and Bürger), Apis (Nelson), Eudemis (Huie), Pieris (Eastham)—the mesh of cytoplasm pervading the yolk now becomes reduced to an almost imperceptible amount owing to a centrifugal flow into the periplasm, which draws in the cleavage-nuclei with it. 'Comet-cells' in fact no longer occur, many of the cells showing, if anything, a tendency to flatten against the periplasm (fig. 27, Pl. 22).

With the entrance of the cleavage-cells into the periplasm the cytoplasms of the two merge into one. Yolk-grains are apt to be carried in with the cells.

There is some uncertainty as to the behaviour of the peripheral cleavage-cells just prior to their entering the periplasm. In Calandra granaria, according to Inkmann, the cells pass into a phase of mitosis with radially directed spindles, the outer daughter-nuclei entering the periplasm, while those at the inner end of the spindle remain as yolk-nuclei. This would be a striking observation if correct; we are, however, unable to confirm it. A stage comparable to Inkmann’s is shown in fig. 26, Pl. 22 (from a 7-hour egg, with 146 cleavage-cells including 20 yolk-cells); it is evident that in only one of the four nuclei drawn is the spindle radial—tangential spindles indeed predominate in the preparation. Inkmann’s conclusion may be tested by determining the relative rate of increase of yolk-
and blastoderm-nuclei at this period. In the egg from which fig. 26, Pl. 22, is drawn there are 20 yolk-nuclei, 8 being in mitosis. Yet in two rather older eggs, with blastoderm already formed, and containing respectively 430 and 700 cells, the number of yolk-nuclei is not more than 45 and 55. The products of cleavage of the peripheral cells have therefore entered the periplasm and not the yolk.

Examination of a large series of preparations at this stage has shown that division of the peripheral cells before entering the periplasm is unusual. More commonly the asters, which are very prominent at this period, only become recognizable at the time of entrance. An example of this is shown in fig. 27, Pl. 22, from an egg with 15 yolk-cells and 90 peripheral cells, while in fig. 28, Pl. 22 (30 yolk-cells, 95 blastoderm-cells), with blastoderm already formed, mitosis is only beginning. Whether the precocious division is completed before entrance into the periplasm, or whether dividing cells are drawn in, is uncertain. There is evidence that the latter is the case; for we have one preparation (30 yolk-cells, 121 peripheral cells) in which the latter, mostly in mitosis, are some within, others without the periplasm—40 have entered (30 of them germ-cells), 61 are still outside, and the remaining 20 are transitional.

Within the newly formed blastoderm it is usual to find almost every cell in mitosis, the spindles being roughly tangential (fig. 29, Pl. 22). The surface of the blastoderm is very irregular owing to protrusion of individual cells. Cell-walls are not yet present.

The completely developed blastoderm is not seen till about the end of the first day. The visible changes that occur during the interval involve (i) a great increase in the number of its cells, the rather loose irregular layer of large flattened or cubical cells giving place to a compact epithelium of narrow columnar cells; (ii) the formation of cell-walls.

At about the fifteenth hour the blastoderm has the following appearance (fig. 30, Pl. 22): the cells have considerably increased in number and decreased in size, and lateral cell-walls have begun to appear, though there is as yet no indication of the internal cell-walls. Internal to the row of nuclei the protoplasm
of the cells is usually rather spongy, and this merges into a gradually thickening zone of exceptionally spongy cytoplasm adjacent to the yolk—the 'secondary periplasm' of Weismann. The latter arises apparently from a local synthesis of protoplasm, and not from a centrifugal flow from the interior of the egg, as Heider (1889) states for Hydrophilus, for the cytoplasm within the egg has already been reduced to an almost negligible quantity.

A later stage of development is shown in fig. 31, Pl. 22. The cells have further increased in number, and are becoming more distinctly delimited from the underlying periplasm, while at the same time an indication of the internal cell-wall appears within the reticular mesh of the secondary periplasm. This cell-wall formation advances, in the entire blastoderm, from behind forwards, the section being drawn from the transitional zone.

The final condition is shown in figs. 32 and 33, Pl. 22. The blastoderm is now a compact columnar epithelium and for the first time has become sharply demarcated by its inner cell-walls from the now gradually increasing cytoplasmic reticulum of the yolk. That portion of the periplasm which has not been absorbed into the blastoderm condenses into a structureless limiting-membrane investing the yolk.

These events do not occur at a uniform rate over the whole surface of the blastoderm, but are more advanced at the hinder than at the anterior pole. This is readily seen in any incompletely developed blastoderm; in Text-fig. 3 b, for example, 18 hours after laying, the cells at the anterior end are larger and more sparse than those in the hinder half—contrast in this respect the mature blastoderm shown in Text-fig. 3 d.

For various insects both synchronous and heterochronous division of the blastoderm-cells have been described. In Calandra a marked synchrony of mitoses undoubtedly occurs in the early blastoderm, entrance into the periplasm being probably the co-ordinating stimulus. Later, however, mitoses are seen at random over the whole blastoderm. The existence of co-ordinating factors, even in later blastoderms, is however shown by the occurrence, at times, of considerable areas of cells all in the same stage of mitosis, with the surrounding cells at rest.
Blastoderm formation. A, newly formed blastoderm; B, more advanced; C, late blastoderm with wave of mitosis (indicated by arrow) passing over it; D, mature blastoderm, with cell-walls visible even in surface view. Germ-cells prominent in A and B; in C they are already level with the surface, but still protrude a little in D.

The most remarkable form of co-ordination encountered in Calandra is seen in the production of a complete ring of mitoses running transversely round the blastoderm. Only two
examples of this have been encountered, both from late blastoderms. One of these has been drawn in Text-fig. 3 c, the ring of mitoses being indicated by the arrow. Every nucleus in the ring is in mitosis, and there is not another visible in the whole blastoderm. Judging by the small size of the nuclei anterior to the ring, a wave of mitosis is passing backwards along the blastoderm.

B. The Yolk-cells. These arise mainly by proliferation of the cells that are left behind in the yolk at blastoderm formation. In many insects re-entrance of blastoderm-cells into the yolk has been described, particularly convincingly in Gryllotalpa (Heymons) and Campodea (Uzel), where they arise exclusively in this way. But beyond the extrusion of an occasional degenerated cell from the blastoderm into the yolk, no evidence for its occurrence in Calandra has been seen. In very young blastoderms cells are occasionally encountered, attached to its under surface, and with radial spindles (fig. 29, Pl. 22). Whether they will later become incorporated into the blastoderm, or will pass back into the yolk cannot be determined. As a source of yolk-cell formation they are unimportant.

Proliferation of yolk-cells during the blastoderm period is very considerable, the 20–30 cells that occur at its inception increasing up to about 700 in the mature blastoderm; these are much diminished in size and are not scattered uniformly through the yolk, but, as described by other authors (Heider, Heymons, Marshall and Dernehl, Carrière, Nelson, Paterson) often form clumps which may contain as many as 20 cells (a few small clumps are seen in figs. 36, 39, Pl. 22).

On the question of the manner of division of yolk-nuclei—whether by mitosis or amitosis—there is much difference of opinion. Cholodkowsky (Phyllodromia), Heymons (Forficula), and Johannsen (Diachrisia) failed to find any indication of mitosis, whereas Heider (Hydrophilus), Eastham (Pieris) and Nelson (Apis) record it, Friedrichs (Donacia) and Marshall and Dernehl (Polistes) describing mitosis in earlier phases, to become replaced later by amitosis. In Calandra mitosis of yolk-cells is commonly seen in early blastoderms (fig. 29, Pl. 22). But in more advanced blastoderms
only occasional preparations show mitosis, but then the majority of cells are in this condition. We have, for instance, one egg with 150 yolk-cells, of which 80, all in the anterior half, are in division; the remainder, in the hinder half, are all at rest. Synchronisation of mitoses, with long intermittent periods of rest, is evidently occurring, mitosis being therefore easily overlooked. Whether amitosis also occurs it is impossible to say.

A feature of these late blastoderms is the large number of degenerate yolk-nuclei which they exhibit. Lecaillon, Friedricks, and Nelson have already referred to these in other insects. Multipolar mitosis has not been observed. A clumping of chromosomes in dividing nuclei, indicating possible degeneration, as described by Nelson for the honey-bee, is frequently seen.

4. THE GERM-CELLS.

These become recognizable at the time of blastoderm formation. They arise, like the blastoderm-cells, by migration of cleavage-cells into the periplasm. They occur at the hinder pole of the egg, but differ from the blastoderm-cells in that they protrude very prominently beyond the surface (Text-fig. 3 A); they are distinguished also by their very rich content of yolk, which they carry with them from the interior of the egg.

As Inkmann has already found for Calandra granaria, a 'Keimbahnplasma' comparable to that described for other insects (v. Hegner, 1914) is not visible.

Proliferation of the germ-cells occurs till there is produced a cluster of still very large cells forming an exceptionally prominent mass at the hinder end of the blastoderm (fig. 30, Pl. 22; Text-fig. 3 B).

At about the end of the first day the germ-cells become withdrawn to the level of the blastoderm surface (Text-fig. 3 c; fig. 33, Pl. 22). By this time they have usually attained that characteristic appearance by which we can readily recognize them in later embryos, the cells being large, with prominent rounded pale nuclei, while the cytoplasm is distinctly eosinophil. Although the yolk-grains are sometimes evident in the germ-cells throughout the embryonic period, they usually soon cease to be conspicuous.
In recent papers—Pierantoni (1927), Buchner (1930), Mansour (1930), Murray and Tiegs (1935)—the peculiar relationship that exists between certain tissues of Calandra oryzae and a bacterial organism has been described. Apart from the specialized bacteria-bearing cells (mycetocytes) that occur in association with the intestine such cells are present also at the tips of the ovarian tubes, whence they infect the eggs, amongst the yolk-grains of which they may be seen. The testis is devoid of mycetocytes.

An association between the bacteria and the germ-cells begins at a very early stage of development. As cleavage progresses the bacteria, hitherto sparsely scattered, begin to accumulate at the hinder pole of the egg, where they become increasingly conspicuous as the germ-cells develop (figs. 30, 32, 33, Pl. 22). In some preparations (haematoxylin staining) they appear merely as an amorphous mass, in others a feltwork of bacteria is visible, while in others again the individual organisms are seen as comparatively large bacteria.

From this mass individual organisms now begin to move out (fig. 30, Pl. 22), and, passing through the secondary periplasm, penetrate into the germ-cells, within which they accumulate in clusters. This occurs irrespective of whether the embryo will become male or female.

The mycetocytes of the ovary develop very early. At the time the germ-cells are becoming withdrawn level with the blastoderm, isolated cells from the latter migrate from the sides into the mass of bacteria (fig. 32, Pl. 22). They are young mycetocytes, and do not appear in the male. Only their nuclei are recognizable, their cytoplasm being entirely obscured by the bacterial content which they acquire.

When later the germ-band develops, the mycetocytes become attached to the mass of germ-cells and thereafter remain in association with them. They are now once more seen as individual cells; sometimes masses of bacteria are still visible within them (fig. 34, Pl. 22), but usually the bacteria pass out of recognition, the cells being distinguishable from the germ-cells only by their deeper staining with haematoxylin, in contrast to the pale vacuolated eosinophil protoplasm of the latter (fig. 60, Pl. 23).
Within the germ-cells, also, the bacteria are usually visible, but are, of course, much sparser than in the mycetocytes. In the male, where mycetocytes are absent, the bacteria mostly remain in the yolk.

5. Formation of Germ-band and Embryonic Membranes.

The earliest indication of differentiation of the germ-band from the blastoderm is seen at the end of the first day; the germ-cells at this period have already become withdrawn to the level of the blastoderm.

Starting in the front half of the egg, and gradually extending backwards, the blastoderm, hitherto of uniform thickness, begins to thin out dorsally owing to its columnar cells becoming gradually flatter. Simultaneously the lateral and ventral walls increase in thickness, the cells becoming irregularly pushed together probably in consequence of the thinning out that occurs along the dorsal surface (fig. 35, Pl. 22). From the thin dorsal part the serosa will form; the thick ventral portion will give rise to the germ-band.

An entire embryo at a slightly later stage of development is shown in Text-fig. 4; a transverse section through the anterior part of the same embryo is depicted in fig. 36, Pl. 22. (Sections towards the hinder end are still similar to that shown in the previous illustration.) In the entire embryo, drawn in lateral
view, the outlines of the germ-band are recognizable, the nuclei showing a tendency to irregular longitudinal alignment, while in the serosa they radiate away from the germ-band. The latter has already grown farther back, being now about three-quarters the length of the egg. The histological structure of the parts is plainly shown in the transverse section; the developing germ-band, it will be noted, has become sharply demarcated from the serosa, while its cells have again formed a regular epithelium.

Text-fig. 5.

Development of dorsal flexure. Note deep invagination of germ-band into yolk at posterior pole of egg (to right). a., amniotic fold.

Differentiation of the germ-band has already begun with the formation of a ventral flattening, the median and two lateral plates thereby becoming recognizable (fig. 36, Pl. 22). It begins very early, and proceeds from in front backwards; in the embryo shown in Text-fig. 4 it has already extended back a third the length of the egg.

The period at which amnion formation begins varies. We have one egg in which its formation has preceded the development of the median plate; while in another the median plate has already completely invaginated to form the inner layer before the amnion has begun to appear. In some embryos (e.g. fig. 37, Pl. 22) the development of the amnion on one side is well advanced before that on the other has even begun. As Heider has already observed for Hydrophilus there is a remarkable lack of synchronization in the development of various parts of the embryo at this early period.
Like the germ-band itself the amniotic folds develop from before backwards. In Text-fig. 4 a fold is just beginning to appear as an inconspicuous ridge at the anterior pole of the egg, more advanced stages of its development being shown in Text-figs. 5 and 6. On either side the folds grow downwards along the junction of the lateral plates and serosa (fig. 37, Pl. 22); it should be noted that the folds are, from the beginning, two-walled, differing in this respect from those of certain Lepidoptera—Pieris (Eastham), Diachrisia (Johanssen). Although mitoses are occasionally seen in the serosa, growth of the embryonic membranes seems to occur chiefly on the thickened zone of insertion of the amnion on to the germ-band.

Fusion of right and left amniotic folds, as they grow downwards under the vitelline membrane, occurs in the mid-line, proceeding as usual from before backwards.
While this portion of the amnion is forming, the germ-band itself is becoming gradually narrower, owing to invagination of the median plate (section 7), till eventually it becomes confined to the lower surface of the egg. The serosa, where it invests the yolk, correspondingly enlarges, while its cells become continually flatter and thinner (cf. figs. 37, 39, and 40, Pl. 22).

At the same time the development of the dorsal flexure of the embryo begins, the method of amnion-formation associated with it being quite different from that seen in the lower half of the egg. The dorsal flexure arises by the germ-band elongating over the hinder pole of the egg on to its dorsal surface. In so doing, however, it does not follow the contour of the egg, but becomes deeply invaginated into the yolk. This is shown in the embryo drawn in Text-fig. 5, while a section through this region, subsequently cut from the same embryo, is drawn in fig. 38, Pl. 22: the invagination is so deep that the embryo at its hinder end is almost crescentic in cross-section; the roof of the invagination is not included in the section for it has been cut rather far to the rear. In later embryos, however, the roof itself extends towards the hinder pole of the egg (Text-fig. 6 A), while the invagination becomes more spherical. The invagination itself, meantime, grows farther along the dorsal surface under the serosa, till eventually at about the twenty-sixth hour it reaches the anterior pole.

The invagination has in the meantime become more dorso-ventrally compressed; it is the amniotic cavity (Text-fig. 6 B; figs. 39, 40, Pl. 22). Its floor, which is very thick, is the dorsal flexure of the germ-band and is much narrower here than along the ventral surface of the egg. The amnion, which forms the roof of the ‘dorsal amniotic cavity’, is much thicker at this period than the ventral amnion, its cells being large and columnar, and, unlike those of the latter, frequently showing mitoses. The serosa which invests the amnion is, as elsewhere, composed of thin flat cells.

As may be expected the deep invagination of the germ-band, as it makes its way forward under the dorsal serosa, is apt to produce at first considerable surface deformation. (The embryo in Text-fig. 5 shows only a little of this.) But the embryonic
membranes soon readjust themselves under the vitelline membrane and any surface distortion soon disappears.

Closure of the amniotic cavity, by fusion of the dorsal and ventral amniotic folds, occurs at the posterior pole of the egg.

In the embryos of many insects a peculiar 'primary dorsal organ' or 'precephalic organ' (Claypole) has been described, of uncertain function and homology. It seems to be very common among the apterygote insects (Lemoine, Claypole, Wheeler, Uzel, Philipschenko), but has been reported also in some of the higher insects—Donacia (Hirschler), Apis (Nelson), Sciara (Du Bois), Corynodes (Paterson). It appears as a dorsal thickening of the blastoderm at about the time of formation of the germ-band. In Calandra no trace of it could be found.

6. SEGMENTATION OF GERMS-BAND AND DEVELOPMENT OF EXTERNAL FORM OF EMBRYO.

A. Segmentation.—In the foregoing account the development of the germ-band has been given to the stage where it has grown up over the hinder pole of the egg, and extended along its dorsal surface to the anterior end. Thereby it attains its maximum length. In some embryos it actually grows down again over the anterior pole of the egg on to the ventral surface.
While these events are in progress, and before the germ-band has yet attained its maximum length, segmentation begins, the segments appearing in regular succession from before backwards,

![Diagram](image1)

**Text-fig. 8.**
Early stage of segmentation, with first two gnathal segments defined. Amniotic folds not yet fused. *h.l.*, head-lobe; *mn.*, mandibular segment; *mx.*, maxillary segment; *v.g.*, ventral groove.

![Diagram](image2)

**Text-fig. 9.**
Embryo with segmentation extending on to beginning of abdomen; antenna and gnathal appendages present, thoracic appendages not yet formed. Amniotic cavity closed (embryonic membranes shown in optical section). *an.*, antenna; *mn.*, mandible; *mx.*, maxilla; *lb.*, labium.

without the occurrence of macro-segments such as have been described by Ayers (1884), Graber (1890), Nusbaum (1889), and Hirschler (1909). The earliest embryo which we have showing indication of segmentation has been drawn in Text-fig. 7; the
head-lobes (protocephalic segment), already evident in Text-fig. 6 A, are clearly defined, and there is an indication of the furrow demarcating it from the mandibular segment.

An embryo at slightly later stage is shown in Text-fig. 8; the head-lobes have become considerably enlarged by spreading up the sides of the yolk, the outlines of the mandibular segment have become better defined, while a faint indentation marks the future labial segment.

In Text-fig. 9 is shown an embryo with now well-defined gnathal segments, while the segmentation behind has extended to the level of the first abdominal segment.

Segmentation of the abdomen is completed at about the end of the second day of development. An embryo in this condition is shown in Text-fig. 10. In the abdomen eleven segments are
to be counted, the last being the largest. In some insects there is definite evidence for the occurrence of a twelfth segment (telson) bearing the anus. It has been observed by Heymons (1895a, 1897a) in the germ-band of Gryllotalpa and Lepisma, while in Carausius Wiesmann (1926) found a diminutive twelfth segment, which became absorbed into the proctodaeum in later embryos. A twelfth segment has also been described by Strindberg (1913) for Eutermes and is stated to occur in certain Hymenoptera—Chalicodoma (Carrière), Apis (Nelson). In Calandra when later the proctodaeum develops this arises not on, but behind, the eleventh segment, and this was also observed by Heymons for Forficula and Periplaneta, while Graber's (1890) illustration of the germ-band of Lina shows the same. A terminal anus-bearing telson must therefore be regarded as proved for the insect abdomen, even though in many it has become reduced to vanishing point (cf. Heymons, 1895b).
By about the sixtieth hour the process of shortening begins, at the completion of which, early on the fourth day, the definitive larval form is recognizable. The process is initiated by a forward movement of the embryo along the ventral surface of the egg, the head-lobes being thereby carried on to its anterior pole (Text-fig. 12); but beyond this slight movement there is nothing corresponding to the remarkable blastokinesis undergone by the embryos of Orthoptera. The condition of the embryo at about the seventieth hour is shown in Text-fig. 13, while in Text-fig. 14 is shown an embryo early in the fourth day, the shortening being now completed while the posterior end of the embryo has reverted to its position at the hinder pole of the egg. The probable function of these remarkable movements is to bring the embryo in contact with as large a surface of yolk as possible.

Text-fig. 12.
Embryo in which oral segment has moved on to front pole of egg. A, nearly ventral view; B, lateral view of same embryo. Labrum prominent; legs enlarged; 10 stigmata; proctodaeum tubular (shown in optical section); amnion no longer separable from serosa.
Meanwhile the germ-band has gradually widened. The expansion of the head-lobes laterally over the yolk has already been referred to. For the post-cephalic segments this occurs much later, and it is not till the stage of shortening of the embryo has set in that it becomes at all active (cf. Text-figs. 10 and 12). By the time the embryo has completed its shortening the yolk has become entirely enclosed at the front and hind poles of the egg, though, in the middle, closure is slower, the egg being at its thickest here.

With the shortening of the germ-band the furrows between
the segments become better defined, and they extend round the embryo as it gradually envelops the yolk.

B. Stomodaeum and Proctodaeum. The stomodaeum is the first to appear. It arises as an oval depression in the middle of the head-segment, usually at the time of formation of the mandibular segment, though in exceptional cases its appearance is delayed till after the formation of the remaining gnathal segments. Later the depression becomes crescentic (Text-fig. 11). When, during the third day the head moves on to the anterior pole of the egg, the stomodaeum is carried with it, and thereby comes to lie horizontally with its opening forwards instead of downwards as in the earlier embryo.

The proctodaeum arises rather later. It develops as a wide, dorso-ventrally compressed ingrowth into the yolk from the hinder end of the germ-band. It usually lies horizontally (Text-fig. 10 b), though in some exceptional embryos, in which the germ-band does not reach the tip of the egg, it grows vertically downwards. Its relation to the germ-band and amniotic cavity is best shown in sagittal sections. Several stages in its early development are shown in figs. 60, 61, 62, 63, 64, Pl. 23. From the beginning the (true) ventral wall is very thick, the dorsal wall on the contrary thin, and merges into the amnion. A sharp line of demarcation between amniotic and proctodaeal cavities

**TEXT-FIG. 14.**

Embryo on fourth day. Embryonic membranes not drawn. Legs still prominent; suture between protocephalic and mandibular segments still distinguishable.
is, in fact, not recognizable at this period, a fact which has led
Inkmann to refer to the latter as 'hinder amniotic cavity'.
Actually it is the lumen of the proctodaeum, the structure to
which he assigns the latter name being the first pair of mal-
pigian tubes (cf. figs. 60-4, Pl. 23).
The proctodaeum arises then, not within the limits of the
eleventh abdominal segment, but behind it. This fact, as al-
ready noted, argues for a terminal twelfth segment, that has
become reduced to vanishing point.
C. The Head and its Appendages.—This develops
out of the head-lobes (protocephalic segment) in which there is
no further external indication of segmentation; and from the
three succeeding gnathal segments, viz. mandibular, maxillary,
and labial.
Of the appendages the mandibular are the first to appear,
followed by the maxillary and labial, the antennae not arising
till rather later. There are no appendages associated with the
intercalary segment. This segment is, in fact, very degenerate
in Calandra, and is distinguishable only by its neuromere
and by an inconspicuous mass of mesoderm. The development
of the labrum is much delayed.
The antennae arise, as usual, as two small backwardly
directed papillae from the postero-ventral region of the proto-
cephalic segment, and as in all insects where their formation
has been adequately studied, are post-oral in position.
The gnathal appendages arise as prominent, rapidly enlarging
laterally projecting outgrowths from the under surface of the
corresponding segments (Text-fig. 9; fig. 44, Pl. 22), the mandi-
bular being the largest.
At about the end of the second day the appendages of the
maxillary and labial segments become constricted into a large
basal and a smaller distal part (Text-fig. 12). The distal lobe
will give rise to the palp; from the proximal part will form the
cardo and the mala. It should be observed that the bilobed
condition arises by transverse constriction, and not, as in some
insects (e.g. Donacia, Hirschler, 1909) by the secondary
addition of a basal part (cardo and stipes) to the distal palp.
Till now the head-segments lie entirely on the ventral surface
DEVELOPMENT OF CALANDRA

of the egg, extending in some embryos nearly to its hinder pole (Text-fig. 9). But with the movement of the embryo above described the protocephalic segment is now carried forward, and so completely occupies the anterior pole (Text-fig. 12).

The latter segment meantime has considerably enlarged, its line of demarcation from the hinder segments being still sharply defined (Text-fig. 12). Of the gnathal segments the mandibular also becomes enlarged. The maxillary and labial however do not share in this enlargement, but, probably owing to invagination of their sternites into the stomodaeum (see below), decrease markedly in size, and become confined to a region ventral to the mandibular segment. The prothoracic segment has meantime also become enlarged, and as the maxillary and labial move into their definitive position, spreads upwards to impinge on the mandibular segment, and eventually almost reaches the hinder margin of the protocephalic. An early stage of this is shown in Text-fig. 12, a later in Text-fig. 13.

It is about this time that right and left halves of the protocephalic segment begin to fuse in the dorsal mid-line, to be followed later by similar fusion in the mandibular segment. The anterior suture of the mandibular segment with the protocephalic becomes increasingly difficult to identify; its position in the definitive head capsule is approximately indicated in Text-fig. 14, and this corresponds fairly closely with that observed by Heymons (1895 b) in Forficula, where the line of fusion is indicated by a well-defined transverse suture demarcating vertex from frons, and is visible even after emergence. The hinder limit of the mandibular segment is impossible to define; it is quite evident, however, that the latter segment gives rise to the greater part of the hinder wall of the head capsule.

By the beginning of the fourth day the head has become well defined, being now sharply demarcated from the thorax (Text-fig. 14). It has become so enlarged by now, that it is to some extent even invaginated into the prothorax, which has, in the meantime, assumed its adult proportions.

There is much variation in time of appearance of the labrum, for while in some embryos it arises before segmentation of the
germ-band is yet completed, in others (e.g. Text-fig. 10) there is no sign of it, even though all the remaining appendages are formed. It arises by the formation of a pair of ridges—the clypeo-labral 'Anlage'—in front of the stomodaeum (Text-fig. 11). The further development is similar to that described by Hirschler for Donacia. Below, the ridges converge on to the stomodaeum. This portion will become the clypeus, while the labrum itself develops from the more distal paired portion, the process involving a reversal in the position of the two. By about the end of the second day, when the protocephalic segment has moved on to the anterior pole of the egg, the labrum has usually enlarged, and projects as a prominent outgrowth beyond the head (Text-fig. 12). Growth of the labrum now occurs in such a way that these paired outgrowths shift into a position below, i.e. oral to, the clypeus 'Anlage', and so come to overhang the mouth (Text-fig. 13 B). At first still paired (Text-fig. 12 A), they soon fuse to a single process (Text-fig. 15).

Owing to change in shape of the protocephalic segment the antennae, now considerably enlarged, have, in the meantime moved on to the sides of the head, and to their definitive position anterior to the mouth (Text-fig. 13 B).1

The position of the mandibles also greatly alters, for they come to lie at the sides of the mouth, undergoing at the same time a rotation in such a way as to direct their free ends towards the mouth (Text-figs. 13, 15).

The maxillae undergo a comparable change, coming to lie postero-lateral to the mouth, with their extremities directed towards it. The formation of the palp by transverse constriction of the appendage has already been referred to. The mala appears to arise as a blunt outgrowth from the basal half. An early stage

1 The post-oral position of the antennary segment is usually accepted by morphologists. Holmgren (1909), however, argues for its pre-oral position, on the ground that the deutocerebral commissure is pre-oral, while the labrum is innervated from the tritocerebral ganglion, and should therefore be part of the third segment. It is difficult to evaluate these facts. The position of the mouth of annelids on the first segment cannot be ignored; while the position of the commissures seems to be a secondary consequence of the final position of the ganglion—e.g. in Scolopendra the tritocerebral commissure is pre-oral, in Scutigera post-oral as in insects.
in the transformation is shown in Text-fig. 15 B—palp and mala are visible, the cardo lying underneath, and therefore not in view. In Text-fig. 15 c the rotation has occurred, all the parts being visible. The structure of the appendage shortly before emergence is shown in Text-fig. 15 D.

The labial appendages meet in the mid-line behind the mouth; the basal parts fuse, the palps becoming directed orally (Text-fig. 15 c, D).

These events are attended by the invagination of the sternites of the gnathal segments into the stomodaeum to form the floor of the mouth, for no part of the head-capsule develops from them.
In Text-fig. 15 A is shown the ventral view of an embryo which has been killed at the time when the mandibular sternites were just undergoing invagination. In Text-fig. 13 A this invagination is complete and the maxillary sternite has advanced to the rim of the mouth. A later stage still is shown in Text-fig. 15 B, the labial sternite being now in course of invagination. When this is eventually completed it allows the labial appendages to approach and unite into the labium (Text-fig. 15 C).

This remarkable dissociation of the sternites from the rest of the segments was first observed by Heymons (1895 b) for Forficula and certain Orthoptera, by Uzel (1898) for Campodea and Tomocerus, and by Holmgren (1909) for Eutermes. In all these cases it gives rise to the hypopharynx. In Calandra, however, where a hypopharynx is absent, the process results merely in formation of the floor of the mouth. In Isotoma according to Philiptschenko (1912) the paraglossae arise in this way.

Chitinization of the head-capsule occurs on the fifth day. A comparison of Text-fig. 15 C and D will show that for maxilla and labium this is attended by considerable shrinkage.

D. Segmentation of the Insect Head.—The difficult task of determining the number of body-segments which, in the extinct ancestors of insects, co-operated in the formation of the complex head-capsule has become a problem mainly for embryology; for the fossil evidence is inadequate, while segmentation in the adult head has become much obscured.

The number of segments involved in the gnathal region may now, with reasonable certainty be taken as three; at any rate, no convincing evidence for the existence of the 'superlingual segment' between the mandibular and maxillary segments is forthcoming. Interest centres, therefore, chiefly in the composition of the large protocephalic segment, i.e. the region anterior to the mandibular segment, the question at stake being whether four or only three segments have entered into its construction.

Commonly it is regarded as composed of three segments, namely an acron (prostomium) fused with (i) the oral segment, devoid of coelomic cavities, with the large protocerebral gang-
lion as its neuromere and labrum as appendage; (ii) the antennary segment, with definite coelom in the embryo, the deutocerebral ganglion being its neuromere, the antenna its appendage; (iii) the premandibular (intercalary) segment, always much reduced in size, with diminutive somite, and with rudimentary appendages surviving in the embryos of primitive forms (cf. Wheeler, 1898; Claypole, 1898; Uzel, 1898; Hoffmann, 1911). It should, however, be observed that the status of the labrum as a true appendage is open to question. Wiesmann's (1926) discovery of mesodermal cavities associated with its 'Anlagen' in Carausius is strong evidence in its favour, as is also its paired origin in many insects. On the other hand, there are species in which it is from the beginning unpaired, a fact which might itself be of no special significance were it not just among the apterygotes that this unpaired origin seems to be general—Lepisma (Heymons, 1897), Anurida (Claypole, 1898), Campodea and Tomocerus (Uzel, 1898), Tomocerus (Hoffmann, 1911), and Isotoma (Philippson, 1912). In Scolopendra also its development as described by Heymons (1901) shows little in common with that of true appendages, for it arises as a median outgrowth from an unpaired clypeus. Its development from paired 'Anlagen' seems therefore to be a secondary acquisition, and, without further evidence, strict comparison with a metameric appendage cannot be unreservedly accepted.

To the three segments above alluded to there must be added, according to Wiesmann's work, a fourth—the reduced pre-antennary, lying to the side of the mouth. Rudimentary appendage-like structures in this region have already been described by other authors; Wiesmann finds, however, that in Carausius there is a coelomic sac associated with each. This discovery appears then to verify Heymons' (1901) conjecture, based on a study of Scolopendra, that the insect head is constructed out of seven, not six segments, as previously held. It is, at the same time, strange that no reference to such a segment has been made for the embryos of apterygotes.

A study of the neuromeres has, so far, failed to give evidence of more than three segments anterior to the mandibular. On
such data Viallanes (1891) based his pioneering study of head segmentation, and most subsequent work has confirmed his observations. The three ganglia of the protocephalic segment of Calandra are shown in Text-fig. 19; a distinct ganglion corresponding to the pre-antennary segment cannot be distinguished, while the ganglion of the oral segment, on the other hand, is greatly enlarged, its three components being clearly demarcated. There is, however, no certain evidence that these lobes are indicative of distinct segments, or that one of them is the ganglion of the abortive pre-antennary segment, for, according to Heymons, they occur also in Scolopendra, where a distinct pre-antennary neuromere is developed in addition. Wheeler (1893), on such evidence, considered the possibility of an additional segment in Xiphidium, but rejected it. Cholodkowsky (1892), however, accepted it for Phyllophormia. The evidence of Scolopendra is, however, decisively against it. Arguments based on the frontal ganglion need not be considered, as this is part of the visceral system.

The homology of the oral segment is uncertain. Heymons regards it as acron, and homologous with the annelid prostomium, the pre-antennary segment being therefore peristomium. It should be observed that, though the first segment is usually designated 'oral' it is really pre-oral, the mouth being intersegmental, for the pre-antennae lie to the side of it. Goodrich (1897) has advanced cogent reasons to prove that the annelid prostomium is not a separate segment, but in front of the first. If this point be conceded it will affect the status of Heymons' first segment. But, in any case, it seems doubtful whether the homology drawn by Heymons is valid. The first segment of annelids possesses, apart from the archicerebrum (lodged in the prostomium) a ventral ganglion, continuous with the ganglionic chain behind. In Scolopendra an archicerebrum is present, the protocerebral ganglia being therefore presumably the equivalent of the ventral ganglia. It seems more reasonable then to regard the first segment as the equivalent of the first (true) annelid segment. Convincing proof of the homology of the labrum with appendages would decide the matter; but on this point further evidence is needed.
While the evidence is, then, still inconclusive it seems that an acron and possibly seven segments, but at least six segments, have entered into the formation of the insect head.

E. Thorax and Abdomen.—The three thoracic segments develop each a pair of backwardly directed appendages, but none form on the abdomen. The legs appear very early, even before the abdomen has completely segmented. They are much smaller than the gnathal appendages (Text-figs. 10, 11, 12). They do not show any sign of segmentation. They remain prominent till the fourth day, but thereafter gradually regress, becoming level with the surrounding body-wall. Here they survive throughout larval life as the imaginal discs from which the legs of the imago will develop.

In the abdomen the number of segments becomes reduced from eleven to ten, due to fusion of the ninth and tenth, on the third day. At the end of the second day the eleventh segment is exceptionally large; but thereafter it becomes reduced in size as the proctodaeum develops at its expense (cf. Text-figs. 13 and 14). It is partially telescoped into the fused ninth and tenth. In the fully grown larva it is very diminutive.

F. Embryonic Membranes.—In their manner of formation, described in section 5, these membranes present nothing unusual. Their later development is, however, peculiar.

Their relationship to the embryo and yolk up to the end of the second day is shown in fig. 59, Pl. 23; the amnion does not exceed the germ-band in width, the yolk being therefore in direct contact at the sides with the serosa. The yolk does not, as in some insects, spread out between amnion and serosa, for it is closely invested by its outer limiting membrane.

Beginning at the hinder pole of the egg and progressing forwards the ventral amniotic cavity now gradually spreads upwards at the sides under the serosa. The amnion becomes thereby stretched into a very delicate membrane, with sparsely scattered nuclei, and adheres usually to the inner surface of the serosa; the inner wall of the enlarging amniotic cavity, scarcely thicker than the amnion, closely invests the yolk. It is a provisional lateral body-wall, and merges below into the thick germ-band (fig. 75, Pl. 24).
On reaching the dorsal surface of the egg the walls of the ventral amniotic cavity now meet and fuse with the dorsal amnion. Dorsal and ventral amnion then merge into one another, and lose connexion with the provisional body-wall, which is itself now merging above into the germ-band. An early stage of this is shown in fig. 88, Pl. 25 (the provisional body-wall is not very extensive here, for the section is through the head-lobes, which cover most of the lateral yolk); a later stage is seen in fig. 75, Pl. 24, and shows the provisional body-wall losing connexion with the amnion. It should be observed that, from their inception, dorsal and ventral amniotic cavities have been in communication at the hinder pole of the egg, and the apparent spreading of the ventral cavity over the sides of the yolk is nothing more than an enlargement of this cavity, which gradually progresses forwards, to form a sac capable of containing the mature embryo, but at a time when the embryo is still in the condition of an elongate germ-band.

At the anterior pole of the egg, between the tip of the proctodaeum and the head-lobes, closure occurs without co-operation of the dorsal amniotic cavity, which is here absent (cf. Text-fig. 10). As Text-fig. 12 b and fig. 65, Pl. 24, show, the provisional body-wall is for a time reflected over the proctodaeal opening, and only later adjusts itself to the surface of the yolk.

The provisional body-wall, then, invests all that portion of the yolk not covered by germ-band; in Text-fig. 13 b, for example, it covers all the part shown as yolk (in the drawing it is indicated only in section at the upper margin). When, during the third day, the germ-band encroaches on the provisional body-wall, it does not displace the latter, but merely incorporates it into itself. Stages in this process are shown in fig. 77, Pl. 24; fig. 115, Pl. 25.

The only noteworthy change in the serosa during these events is the formation of a delicate chitinous sheath on its exterior (cf. figs. 75, 77, Pl. 24; fig. 88, Pl. 25). A similar sheath has been described by Heymons in Lepisma (1897 a). The amnion is exceedingly inconspicuous and usually adheres to the serosa. The embryonic membranes survive thus throughout embryonic life, and must apparently be ruptured by the embryo.
The spreading of the provisional body-wall over the surface of the yolk recalls in some respects Strindberg's (1913) account for Chrysomela; in that insect, however, it becomes displaced by the encroaching germ-band, and degenerates in the yolk as a 'dorsal organ'. Such an organ, indeed, does not form at all in Calandra, which in this respect resembles certain Lepidoptera (cf. Ganin, 1870; Hirschler, 1928; Eastham, 1930 a), where the embryonic membranes survive till emergence of the embryo. In Bombyx according to Ganin the embryo devours the membranes.

7. DIFFERENTIATION OF THE GERM-BAND INTO OUTER AND INNER (LOWER) LAYERS

The early phases of this are described in section 5.

Inner layer formation begins by invagination of the median plate along the greater part of the length of the embryo. Its anterior limit is at the site of formation of the future stomodaenum; while posteriorly it extends as far as the mass of germ-cells, which has become overgrown by the hinder part of the germ-band. The invagination occurs by a bending in of the median plate (fig. 37, Pl. 22), and as this progresses its cells become completely separated off from the lateral plates, which close in beneath it, as the outer layer (figs. 38, 39, Pl. 22). In all embryos examined these events were initiated at the anterior end of the germ-band. The mass of cells thus invaginated is from the beginning a solid cord and is devoid of any trace of a cavity as described for some insects—Hydrophilus (Kowalewsky, 1871; Heider, 1889), Donacia (Hirschler, 1909).

A longitudinal furrow (gastral or ventral groove) marks the line of invagination and of incomplete fusion of the lateral plates; it is seen in Text-figs. 7 and 8, and in figs. 37, 38, Pl. 22. This groove is, however, not long-lived, for the lateral plates soon close in completely under the invaginated mass. At a later period another groove appears in the same place in the outer layer, but this is the neural groove, whose formation is associated with development of the nerve-cord. It is seen in Text-figs. 10–13.

There is much variation in the synchronization of events at this period of development. In some embryos inner layer
formation does not begin till well after the amnion has started to develop; in others again invagination may be complete before there is yet a sign of amnion. In Inkmann’s account for Calandra granaria the development is described of a ‘ventral groove’ preceding amnion formation, which then is said to disappear again, the inner layer arising concurrently with the amnion as a median proliferation of cells, and not as an invagination of a median plate. Unless Calandra granaria is, in this respect, different from Calandra oryzae, Inkmann’s ‘ventral groove’ is merely a case of precocious inner layer formation.

These events occur, and may often be completed, before the dorsal flexure of the embryo has begun to form. The inner layer associated with the dorsal flexure is considerably more massive than that along the ventral surface (figs. 39, 40, Pl. 22), the latter, however, thickening towards the hinder pole of the egg.

Inner layer formation is attended by thickening of the lateral plates. This arises from two causes (i) an elongation of its cells, due chiefly to their developing large vacuoles, (ii) a tendency to form more than one cell-layer.

The cells of the median plate are, like those of the lateral plates, vacuolated and columnar. After invagination they become rounded or polygonal, but retain the vacuoles for a time. Mitoses are now frequently seen among them. As far as could be determined this local cell proliferation alone is the cause of the subsequent enlargement of the inner layer, there being no indication of an acquisition of cells from the lateral plates as described for other species.

The inner layer very early acquires an ill-defined segmentation, consisting of a succession of irregular thickenings, due to local piling up of cells, which correspond in position remarkably with the site of the future body-segments (fig. 41, Pl. 22). In some embryos, however, it is not seen. Precocious segmentation of the inner layer has already been described by Heider for Hydrophilus and by Graber and Eastham for Pieris. In both these species a lateral segmentation is involved, of a kind which does not occur in Calandra. Here the inner layer can readily be examined in its entirety in cleared embryos. It is
much narrower on the dorsal flexure than elsewhere, but only minor indentations appear along its lateral margins, and there is no sign of lateral segmentation.

The cells of the inner layer now begin to spread outwards over the surface of the outer layer, to which they eventually form an almost complete lining. Early stages in this spreading are seen in figs. 40 and 42, Pl. 22; the cells, it will be seen, present no regular form or arrangement. But in the more advanced stage shown in fig. 43, Pl. 22, they have become consolidated into a regular epithelium. Active cell-division accompanies the spreading. Both in the inner and outer layers vacuoles now cease to occur.

The outer layer is, by almost general consent, ectoderm; the interpretation of the inner layer has given rise to much discussion. Heymons, who derives the mid-gut in the pterygote insects from stomodaeum and proctodaeum, regards it as mesoderm, and identifies the endoderm with the yolk-cells. More commonly, however, it is considered as ‘mesendoderm’, on the ground that the mid-gut also arises from it, either along its whole length, or from cells localized in the ‘endoderm rudiments’ at its anterior and posterior ends; the yolk-cells are then described as a ‘parablast’, and not a true germ-layer. The theoretical discussion will be left to a later section (9 c); here we confine ourselves to matters of direct observation.

Now in Calandra, the mid-gut has a bipolar origin, and arises not from anterior and posterior endoderm rudiments (for these do not occur) but, as Mansour (1927) first showed, from the stomodaeum and proctodaeum (section 9 b). The inner layer therefore plays no part in its development. Mansour identifies the endoderm not with the yolk-cells, as does Heymons, but with certain cells that are said to migrate from the inner layer into the yolk and there degenerate. As an important theoretical point is involved, we have examined the matter carefully in numerous serial sections of embryos at appropriate age, but have been unable to find evidence for passage of such cells into the yolk. Mansour writes that the cells ‘form a syncytium with deeply staining constituents’. Such cells undoubtedly occur in large numbers in the yolk, even before the inner layer
is formed, but are the clusters of yolk-cells already described (section 3 B), and are readily distinguishable from the cells of the inner layer, for these are, in contrast, rounded, vacuolated, and rather hyaline, and quite different in appearance; nor are transitional stages between the two types of cell found. Apart, therefore, from the impossibility of obtaining evidence for identifying them with endoderm, we must deny the occurrence of such cells in Calandra.

Inkmann (1933) derives the mid-gut from the innermost cells of the inner layer along its whole length, and regards them as endoderm; the histological features by which he distinguishes these cells is, in our material, anything but constant, and in any case it is quite evident that he has altogether misinterpreted the process of mid-gut formation in Calandra.

There is then no adequate reason for identifying any part of the inner layer of Calandra with endoderm.

8. Differentiation of the Inner Layer, up to the Formation of the Coelomic Sacs

A. Segmentation.—During the period of lateral spreading of its cells, the early segmentation of the inner layer, described in the last section, becomes temporarily obscured, and does not reappear till the cells have become ordered into a regular epithelium. This occurs near the middle of the second day, the embryo being at about the stage of development shown in Text-fig. 9. It appears first in the thorax and the labial segment, and spreads backwards along the abdomen, slightly preceding the external segmentation.

This segmentation results in the formation of two lateral rows of somites, separated by a thin median unsegmented strip of mesoderm (figs. 48, Pl. 22; fig. 59, Pl. 23). In the latter the cells form a simple epithelium, one cell thick. In the somites two layers occur—the more internal (splanchnic) layer comprising at first rather flattened cells which multiply and later become cubical; while adjacent to the ectoderm the somatic layer is formed of cells which become gradually more elongate (fig. 45, Pl. 23). At the intersegments the lateral zones are reduced to one cell in thickness (fig. 59, Pl. 23).
In rather later embryos a complete separation of successive somites may be effected (fig. 45, Pl. 23). For Orthoptera this condition is well known; for Coleoptera it seems to be unusual.

The somites lie towards the outer margin of the body-wall, dorso-laterally to the appendages when these are present (figs. 46, 47, Pl. 23).

In the abdomen segmentation extends to the hinder end of the ninth segment. A somite is present in the tenth (fig. 61, Pl. 23), but though demarcated from the ninth is confluent behind with a prominent and still enlarging unpaired mass of mesodermal cells situated in the last segment, behind the germ-cells and occupying much of the space between the proctodaeum and the body-wall (figs. 61, 62, Pl. 23).

In the maxillary and mandibular segments somites do not occur, the lateral zones of mesoderm being not even epithelial in character. In the mandibular segment this mesoderm is much reduced (fig. 44, Pl. 22). In the region of the intercalary segment it is impossible to distinguish a somite; later, however, there is an indication of a vestigial coelomic sacs (v. section 14).

Owing to the greater width of the germ-band in the gnathal region the layer of mesodermal cells is apt to be rather thinned out there, this being accentuated by the mesoderm extending as a loose clump of cells into the cavities of the appendages; the antennae, which are post-oral in position at this period, also acquire mesoderm in this way (fig. 59, Pl. 23).

With the subsequent enlargement of the gnathal appendages, particularly the mandibular, these loose clumps of cells become converted into simple epithelial linings for the appendages (fig. 44, Pl. 22). Closed sacs do not form.

At this period, then, the mesoderm of the gnathal segments consists of a thin sheet of cells, slightly thickened laterally, but much reduced in the mandibular segment, with pocket-like extensions into the appendages (particularly mandible), and with a pair of somites in the labial segment.

B. The Pre-oral Mesoderm.—This has, as in all higher insects, a post-oral origin. While the cells of the inner layer spread out over the ectoderm, a small clump of cells remains heaped up immediately behind the developing stomodaenum.
(figs. 52, 53, Pl. 23). This must not be taken for 'anterior endoderm rudiment', for it plays no part in mid-gut formation.

From this clump cells now spread forwards round the stomodaeum, and while undergoing active division extend in the form of two sheets laterally along the floor of the protocephalic segment. These two strands of mesoderm become united by a transverse band of cells in front of the stomodaeum, which in this way becomes encircled with mesoderm.

During the latter half of the second day the cavities of the paired labral 'Anlage' have become invaded by cells that migrate from the mesoderm in front of the stomodaeum.

By the end of the second day the pre-oral mesoderm has developed into a consolidated layer of cells on the floor of the protocephalic segment, spreading at the same time, as a layer of scattered cells, up the sides. It remains connected with the post-oral mesoderm by a pair of thick bands to the side of the stomodaeum, representing the premandibular (intercalary) mesoderm. The mesoderm associated with the antenna, which has begun now to move forwards, is very conspicuous.

C. The Coelomic Sacs.—During the second day the somites have enlarged, cell-division being frequently encountered. The coelomic sacs arise towards the end of the second day, their formation being initiated by the appearance of a narrow cleft between the splanchnic and somatic layers.

Probably owing to secretion of fluid into their interior these clefts now expand into prominent cavities. They develop in the somites of the labial to ninth abdominal segments. In the tenth abdominal somite a cavity does not appear, nor does it form in the mesoderm of the mandibular and maxillary segments. In the thorax and the labial segment the coelomic sacs are elongate and oval; in the abdomen when fully developed they are rather more spherical.

The separation between successive somites above alluded to is now no longer seen. Early on the third day, at a time when the germ-band is beginning to shorten, the cavities of successive somites communicate with one another by narrow though well-defined channels in the intersegmental constrictions (fig. 48, Pl. 28), so that the coelomic sacs now form a pair of moniliform
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rubes running from the labial to the ninth abdominal segment. While this condition is well known for Orthoptera and Dermaptera, there is some doubt as to its occurrence in Coleoptera. Hirschler (1909) was unable to detect it in Donacia; it has, however, been described by Graber (1890) for Melolontha and Lina, and by Heider (1889) for Hydrophilus. With the use of celloidin embedding, as in the present work, the tendency to form artificial cavities within the tissues is reduced to a minimum.

In the protocephalic segment only one pair of coelomic sacs forms, namely in association with the antennae. They appear later than do the others; not indeed till after the germ-band has begun to shorten, i.e. early on the third day. Although the mesoderm of the antenna has not hitherto shown the characters of a true somite, the coelomic sac which forms within it does not, except for its later appearance and rather small size, present anything unusual. It does not occupy the cavity of the antenna, but lies at its base (fig. 86, Pl. 25), in the main a little to the rear of it (fig. 87, Pl. 25). A premandibular coelomic sac does not form (see further, section 14). In the labrum, also, coelomic sacs do not appear, there being indeed no evidence for the formation even of somites in association with it (cf. fig. 92, Pl. 25); this is noteworthy because Wiesmann (1926) has described definite coelomic sacs in the labrum of Carausius.

The coelomic sacs of Calandra closely resemble those observed for other members of the higher orders of insects, there being nothing comparable to the large, often trilobed cavities, with extensions into the appendages, that are described for the more generalized forms—Orthoptera (Cholodkowsky, 1889; Graber, 1890; Heymons, 1895; Wiesmann, 1926), Forficula (Heymons, 1895), Lepisma (Heymons, 1897 a), Eutermes (Strindberg, 1913); as well as in Scolopendra (Heymons, 1901).

The occurrence of coelomic sacs in Calandra from the labial to the ninth abdominal segment agrees well with Hirschler’s description (1909) for Donacia. For the head segments, however, an important difference is to be noted. According to Hirschler there is a well-developed intercalary coelom, from which the cephalic aorta arises, while an antennary coelom
is absent. This is surprising, in view of the fact that an anten-
nary coelom is almost universal in insects (it seems to be absent
in Hydrophilus and Pieris), while an intercalary coelom
occurs, even as a vestige, in only the most primitive forms.
Since Hirschler derives the sub-oesophageal body (q.v.) from the
mid-gut epithelium, and not, as usual, from the intercalary
mesoderm, it seems very probable that he has misinterpreted
this region of the embryo. It is desirable that Donacia
should be reinvestigated on this point, as it forms the type for
current text-book description.

9. ALIMENTARY CANAL: EARLY DEVELOPMENT.

A. Early Development of Stomodaeum and Proctodaeum.—This has already been alluded to in the
general description of the germ-band (section 6 B). In the pre-
sent section their early development only is described; for the
later phases see section 11.

(i) Stomodaeum.—In very early germ-bands, at a time
when the dorsal flexure has scarcely attained its maximum
length, the ectoderm presents a pronounced thickening just
anterior to the tip of the invaginated inner layer, a thickening
which appears accentuated by the fact that the ectoderm im-
mediately behind it is unusually thin (fig. 49, Pl. 23).

At a rather later period, when the gnathal segments have
already appeared, this thickened area is seen in process of
invagination; it is the ‘Anlage’ of the stomodaeum (fig. 50,
Pl. 23). Becoming later hemispherical (fig. 51, Pl. 23) it then
gradually elongates and becomes more and more cylindrical
(fig. 52, Pl. 23).

Even before invagination of the stomodaeum has begun, a
slight heaping up of mesoderm cells at the tip of the inner layer
is seen. This has already been alluded to in the foregoing
section, and must not be mistaken for ‘anterior endoderm
rudiment’. During the subsequent migration of its cells round
the stomodaeum some of them spread on to its postero-lateral
wall; but except for this, the stomodaeum remains mainly in
direct contact with the yolk.

(ii) Proctodaeum.—In section 6 B the development of the
proctodaeum has been described to the stage at which, in the latter half of the second day, it occurs as a horizontal cleft-like ingrowth into the yolk, its (true) ventral wall being thick, but its dorsal wall at this period scarcely thicker than the amnion itself with which it is continuous. Its appearance in sagittal section is shown in figs. 60, 61, Pl. 23; while fig. 62, Pl. 23, represents a transverse section.

Towards the end of the second day the dorsal wall becomes much altered; its cells multiply considerably, and become long and cylindrical, and the wall therefore much thickened (figs. 63, 64, Pl. 23). It grows also considerably in length and extends forwards almost to the level of the eighth abdominal segment.

The relation of the proctodaeum to adjacent parts at about the end of the second day is shown in fig. 63, 64, Pl. 23. Within the eleventh segment the inner layer is very thick and forms a large unpaired mass occupying all the space between the proctodaeum and the body-wall, and separated from the yolk by the still unpaired mass of germ-cells. The first pair of malpighian tubes is beginning to form. The cavity of the proctodaeum has not yet opened on to the yolk.

Till now the proctodaeum is still dorso-ventrally compressed. But a change now occurs whereby it becomes converted into a cylindrical tube, of which the lumen is now sharply distinguishable from amniotic cavity (figs. 65, 66, Pl. 23; fig. 88, Pl. 25). At its most internal extremity, however, where it abuts on the yolk, its roof remains thin, and its cavity dorso-ventrally flattened (fig. 67 B, Pl. 24).

B. The Mid-gut.—The development of this organ still remains one of the most controversial problems of descriptive embryology. The literature on the subject is treated at length by Hirschler (1928) in Schröder’s ‘Handbuch’ and in the special review by Eastham (1930 b). For present purposes the following short summary will suffice:

(i) The mid-gut is formed from the yolk-cells. Although held by many of the older writers, this view now finds support only in Heymons’ work on Lepisma (1897 a) and Campodea (1897 b).

(ii) The mid-gut is a derivative of the proctodaeum and
stomodaeum, and is therefore ectodermal. This view originated, it seems, with Ganin (1874) and was upheld by Graber (1890, 1891) for Orthoptera, and Korotneff (1894) for Lepidoptera. With the appearance of Heymons' great work on the Orthoptera and Dermaptera in 1895 the subject first gained prominence, and has since found support in the work of Schwartze (1899) and Johannsen (1929) on Lepidoptera, and of Lecaillon (1898), Deegener (1900), Friedrichs (1906) and Mansour (1927) on Coleoptera.  

(iii) The mid-gut cells arise either by delamination, or invagination, or inward proliferation from the outer layer, in complete independence of stomodaeum and proctodaeum, and by a process which is usually regarded as, at least in principle, comparable with gastrulation. In one form or another most authors support this view.

In Eutermes and several Hymenoptera and Coleoptera such endodermal cells are, according to Strindberg (1913) spread throughout the length of the inner layer; and a similar conclusion is reached by Hammerschmidt (1910) and Leuzinger and Wiesmann (1926) for Carausius, by Inkmann (1938) for Calandra and by Paterson (1935) for Corynodes. Anterior and posterior endoderm rudiments are said, then, not to occur.

For most species, however, the bipolar origin, first described by Kowalewsky (1886) for muscids is observed, though some authors admit the participation of a narrow median connecting strand ("Mittelstrang")—Nusbaum and Fulinsky (1906, 1909), Hirschler (1909, 1912), Philiptschenko (1912).

In regard to the origin of the bipolar rudiments, these are said to arise either (i) as thickenings of the inner layer—Kowalewsky (1886) for muscids, Heider (1889) for Hydrophilus, Wheeler (1889) for Blatta and Doryphora, Strindberg (1914) for Vespa, or (ii) as an independent inward proliferation of cells from the outer layer—Carrière and Bürger (1897) for Chalicodoma, Noack (1901) for Calliphora, Nusbaum and Fulinsky (1906, 1909) for Phyllodromia and Gryllotalpa, Nelson (1915) for Apis, ?Strindberg (1916)

1 Roonwal (Phil. Trans. Roy Soc. B.227.1937) upholds this for Locusta. This paper appeared too late for reference in the text.
DEVELOPMENT OF CALANDRA

for Sialis, Eastham (1929) and Henson (1932) for Pieris, Mellonby (1935) for Rhodnius, and Thomas (1936) for Carausius.

It would be an error to regard these diverse views as mutually exclusive, for they relate to insects of the most varied kinds. But in some cases, where identical or closely related species are concerned, comparison is possible. Thus Hirschler’s interpretation of mid-gut formation in Donacia differs radically from that of Friedrichs on the same species; while in Carausius the recent account by Thomas is impossible to reconcile with that of Hammerschmidt, and of Leuzinger and Wiesmann.¹

In regard to Calandra Tichomirow derives the mid-gut from yolk-cells, Mansour from stomodaeum and proctodaeum, while Inkmann describes its origin from endoderm cells distributed throughout the length of the inner layer. Our own observations agree essentially with those of Mansour.

(a) Anterior (stomodaeal) Component of Mid-gut.—If an embryo from early in the third day be examined, i.e. at about the stage shown in Text-fig. 12, the mid-gut ‘Anlage’ is clearly recognizable as far back as the level of the labial segment, where it is seen as a pair of narrow lateral bands lying on the coelomic sacs next to the yolk (fig. 47, Pl. 23). Anteriorly to this the bands widen, forming, just behind the stomodaeum, a complete sheet of cells under the yolk. The cells are usually rather large, pale, vacuolated, and well merit the name ‘succulent’ that is often applied to them. In rather younger embryos the hinder limit of the mid-gut ‘Anlage’ may be seen extending to various levels between the stomodaeum and the labial segment. In some embryos at about this period it is represented only by a transverse bar behind the stomodaeum (fig. 59, Pl. 23). Since there is no evidence for a local differentiation of the cells of the mid-gut ‘Anlage’ from the coelomic sacs, it is evident that there must be occurring a backward migration from the region of the stomodaeum, and it remains to determine the exact origin of its cells. The difficulty

¹ The structure which Thomas regards as posterior endoderm rudiment seems to be the genital rudiment (cf. fig. 13 of Thomas with fig. 6 (p. 142) of Wiesmann).
which has been experienced in elucidating this point reflects
the sharply contrasted opinions concerning it which are expressed
in the literature. The following account is based on an examina-
tion of numerous carefully prepared celloidin sections, in which
the fixation and staining left little to be desired; the accompan-
ying illustrations have been drawn, cell for cell, with scrupulous
accuracy, and with use of the camera lucida.

The condition of the stomodaeum, immediately before the
mid-gut ‘Anlage’ appears, is shown in fig. 51, Pl. 23 (sagittal
section). It is now a hemispherical organ, with pronounced
lumen; there is a slight heaping up of inner layer cells just behind
it, while others have migrated round, and now appear in front
of it. Incidentally, it may be noted, Paracytoid formation
(v. section 21) is very active.

Till now the hinder wall of the stomodaeum has preserved its
epithelial character. Mid-gut formation is initiated by some of
these cells now elongating and extending outwards towards the
yolk. A very early stage of this is shown in fig. 52, Pl. 23; the
stomodaeum has become cylindrical, the epithelial character of its
hinder wall disorganized, and some of the cells have grown long
and narrow, but are hardly yet protruding beyond its surface.

A later stage is shown in fig. 53, Pl. 23; the hinder wall of the
stomodaeum has now completely lost its epithelial character;
it has grown markedly in thickness, and its cells are evidently
growing backwards over the heap of inner layer cells.

In fig. 55, Pl. 23, a rather later stage still is shown. The mid-
gut ‘Anlage’ now appears as a compact mass of cells, still con-
nected in front with the hinder wall of the stomodaeum, whose
regular epithelial structure is here disorganized. The cells have
already begun to assume the peculiar texture of mid-gut cells.
The ‘Anlage’ itself is sharply demarcated from the underlying
inner-layer cells. It is important to observe that there is no
evidence for regarding it as a differentiation of the invaginated
inner layer; nor does it arise, as in Chalicodoma (Carrière
and Bürger, 1897) by proliferation of the outer layer, which
then invaginates with the stomodaeum, but from the stomo-
daeeum itself. That the inner layer plays no part in its formation
is well shown in those embryos where it arises from a more
restricted part of the stomodaeal wall; fig. 54, Pl. 23, will render
detailed description unnecessary.

From the mid-gut 'Anlage' which thus arises from the hinder
wall of the stomodaeum, cells now spread forwards to the sides
of the stomodaeum, over the mesoderm cells, and become the
pre-oral mid-gut 'Anlage'. This is well seen in fig. 57, Pl. 23.
The drawing is from an embryo cut in horizontal section. The
stomodaeum is cut transversely; its hinder wall has lost its
epithelial character and the mid-gut cells are seen spreading
laterally and then forwards round the stomodaeum. The pre-
oral mid-gut cells are to be seen also in fig. 64, Pl. 23. From
them forms that part of the mid-gut wall that lies just dorsally
to the oesophagus.

In embryos in which the coelomic sacs have begun to open,
the mid-gut 'Anlage', though still forming a prominent mass of
cells just behind the stomodaeum, has now lost direct connexion
with the latter, the posterior wall of which has regained its
regular epithelial character (fig. 56, Pl. 23). Backward migration
of the mid-gut cells, which has already been described, now
begins. It is attended by much cell-division. The post-oral mass
thereafter gradually dwindles.

(b) Posterior (proctodaeal) Component of Mid-
gut.—This develops much later than the stomodaeal com-
ponent, for it does not arise till after the germ-band has begun
to shorten, i.e. early on the third day, and at a time when the
stomodaeal component has already grown back as far as the
first thoracic segment.

The blind end of the proctodaeum widens out and begins
to bend down over the germ-cells. Simultaneously the dorsal
(thin) wall of the proctodaeum opens, thereby giving the yolk
direct access to the lumen of the proctodaeum. This early
opening of the proctodaeum is noteworthy, since later it again
becomes completely closed. Though having direct access to the
proctodaeum, the yolk does not, as a rule, enter it, being held
back by its limiting membrane.

From the thick ventral wall of the proctodaeum, as it bends
over the mass of germ-cells, there grow out two lateral strands
of cells which, insinuating themselves between the yolk and the
germ-cells, eventually extend along the mesoderm to the eighth abdominal segment. The median zone of the proctodaeum grows more slowly, but soon it, too, grows down on to the mesoderm, the germ-cells being thereby completely cut off from contact with the yolk. In this way is formed the posterior 'Anlage' of the mid-gut.

As a possible origin of mid-gut from proctodaeum has been the subject of so much discussion it is desirable to illustrate the evidence for it as completely as possible. In fig. 67 A–H, Pl. 24, is shown a series of successive transverse sections of the proctodaeal region of an embryo in which shortening of the germ-band is just beginning (cf. Text-fig. 12). The tubular proctodaeum (cf. fig. 83, Pl. 25) is shown widening out in fig. 67 A, Pl. 24; the roof has become thin, the first pair of malpighian tubes is seen in transverse section, and the base of the second pair is just distinguishable. As the tip of the proctodaeum is approached in the succeeding sections, it is seen gradually widening out. In C and D disappearance of the thin roof is observed, giving thereby direct access of the yolk to the lumen of the proctodaeum. In the succeeding sections the median portion of the ventral wall gradually thins out and disappears, while thickened strands from its lateral margins extend as mid-gut 'Anlagen' as far as the eighth abdominal coelomic sac in figs. 67 a and h, Pl. 24. (Note ninth coelomic sac in C and D.)

The embryo here selected shows the hinder mid-gut 'Anlage' at the very beginning of its formation. The ensuing events may be pictured by reference to fig. 68, Pl. 24. This is from a rather later embryo, the section passing through the eighth abdominal coelomic sac (i.e. at about the same level as fig. 67 a, Pl. 24). The mid-gut 'Anlage' is now a complete sheet of cells shutting off the germ-cells from the yolk.

The divergence of the mid-gut 'Anlagen' from the tip of the proctodaeum, as here described, is best studied in transverse section. Sagittal sections are, however, very instructive. Fig. 64, Pl. 23, shows such a section from an embryo in which the proctodaeum is at a rather earlier stage of development than that shown in the serial section, for it is still a dorso-ventrally compressed cleft. It should be compared with the stage shown in
fig. 63, Pl. 23, where there is yet no sign of mid-gut formation. From the tip of the proctodaeum a few cells are seen spreading over the germ-cells, reaching the level of the eighth abdominal coelomic sac. At the same time the opening of the proctodaeum on to the yolk, which is later so conspicuous (fig. 65, Pl. 23), is just becoming apparent.

Taken by itself this section will not give an adequate picture of these early stages of mid-gut formation; but in conjunction with the transverse sections depicted a clear idea of events will be obtained.

Anterior and posterior mid-gut 'Anlagen' meet, towards the end of the third day, at about the level of the first or second abdominal segment. Although each extends through about seven segments the anterior is much the larger, owing to the greater size of the anterior segments.

C. Discussion.—Before discussing the bearing of these observations on the germ-layer theory it is desirable to comment on their objective validity.

(a) Validity of the Observations.—The difficulty which has confronted most observers on this point is to decide whether the mid-gut cells arise from stomodaeum and proctodaeum, or from the immediately adjacent inner-layer cells.

In the case of the proctodaeal component this difficulty does not arise in Calandra, for the tip of the proctodaeum is, from the beginning, separated from the inner-layer cells by the large mass of germ-cells; as fig. 61, Pl. 23, will show, the large mass of inner-layer cells in the eleventh segment cannot possibly participate in mid-gut formation.

For the stomodaeum the matter is not so simple, there being a close association between it and the anterior clump of inner-layer cells. Is the latter an 'anterior endodermal rudiment', or is it purely mesoderm? MacBride (1914) commenting on the point writes that it is a case 'where the endoderm rudiment becomes distinguishable at a very late stage of development, and where its first origin is impossible to determine with accuracy'. This difficulty was encountered during the present work, and preliminary observations, based on the study of transverse sections, appeared to confirm the orthodox view.
The difficulty with the transverse sections is that the disorganization of the hinder wall of the stomodaem cannot be detected by their means, the cells which migrate backwards over the piled-up mesoderm having the appearance of originating from the latter. If, for example, the embryo drawn in fig. 53, Pl. 23, had been transversely cut, there would have been no clue to their origin from the stomodaem, and they would have been interpreted as a differentiation from the inner-layer cells. Moreover, in transverse sections the mid-gut cells usually appear sharply demarcated from the stomodaem, particularly from its lateral walls (fig. 58, Pl. 23); but this is because they have migrated there from the hinder wall. Reference to fig. 57, Pl. 23, will make this clear.

In sagittal sections, however, the origin of the mid-gut rudiment as an outgrowth from the hinder wall of the stomodaem itself becomes apparent. The temporary derangement of its epithelial wall, and the backward migration of cells from this part over the heaped-up mesoderm is then obvious, and there is no evidence for a local differentiation from the latter.

To make the demonstration of this very controversial point as objective as possible, three photographs are here offered (Text-fig. 16 A, B, c), representing the same objects as have been drawn in figs. 50, 52, and 55, Pl. 23. In c the mid-gut ‘Anlage’ is very conspicuous; it is directly continuous with the hinder wall of the stomodaem, and is sharply demarcated from the inner-layer cells, over which it is growing. Has it arisen from the outer layer and then become drawn in with the stomodaem as the latter invaginated? Fig. A shows that this is not the case, for there is no indication of it yet, although the stomodaem has itself become defined. Even in B, with the stomodaem already cylindrical, it is only just beginning to form, its site of origin being the tip of the stomodaem.

No claim is made for the generality of this observation, for the opposite conclusion of Nusbaum and Fulinsky (1906) on Phyllodromia germanica, and of Henson (1932) on Pieris brassicae seems equally conclusive for those species. The former work is of special interest because it employs one of the species on which Heymons based his novel views. Nus-
baum and Fulinsky, and Henson both describe a proliferating zone of ectoderm immediately behind the place, or, in Pieris, at the place, where the stomodeum will later form. From this zone of proliferation both mid-gut rudiment and some meso-

dermal cells arise. In Phylodromia this zone of proliferation may become invaginated with the stomodeum, and so give the false impression of actually arising from the latter. In this way the orthodox view of the mid-gut arising from a kind of blastopore, i.e. common meeting-ground of ectoderm, mesoderm, and endoderm, may perhaps be upheld, while the need of deriving it from the stomodeum, i.e. conventional ectoderm, is avoided. But for Calandra this explanation will not hold; such a zone of proliferation does not occur here and the mid-gut forms from the stomodeum itself.
(b) Application to the Theory of Gastrulation, and the Germ-layer Theory.—It is evident that the foregoing observations on the origin of the mid-gut of Callandra involve the validity of the theory of gastrulation and of homology of germ-layers. Heymons has already discussed the implication of such observations in his work on the Dermaptera and Orthoptera (1895) and in his later monograph on Scelopendra (1901); but although the validity of his discussion is open to question it is the observations themselves and not their consequences alone which have been often discredited. Having encountered in Callandra an insect whose development conforms with that described by Heymons, the need arises for again considering the meaning of such observations, particularly as much of the past discussion on the subject is marred by the fact that the principles involved have acquired a variety of meanings with different authors.

In his celebrated 'Studien zur Gastraea Theorie' Haeckel, to whom the term is due, defines the gastrula as a 'monaxial unsegmented hollow body, without appendages, whose simple cavity (Urdarm) opens by an orifice (Urmund) at one pole of the axis, and whose body-wall is composed of two layers of cells—endo derm or gastric layer, and ectoderm or dermal layer'. Its importance lies in the fact that it occurs 'in animals of the most diverse classes, from sponges to vertebrates in the same characteristic form'; and in the special significance which Haeckel attached to it as recapitulating in the ontogeny of the individual the extinct gastraea ancestor of the metazoa. Four varieties of gastrula were described, the insect gastrula belonging to the type designated 'perigastrula'. An illustration, supposedly based on Kowalewsky's (1871) account for Hydrophilus, accompanies the description and shows the invaginating ventral groove designated endoderm, with its cavity the 'Urdarmöhle'. But to conform to the scheme this gastrula has been constructed in disregard of Kowalewsky's observation that the invaginated layer is not endoderm, nor its cavity an archenteron.

Balfour (1880) showed the error of this interpretation and denied the existence of a gastrula in the ontogeny of insects. The results of subsequent investigation have vindicated his
view, for the ventral groove is a secondary acquisition of higher insects, being absent in *Scolopendra* and in all the apterygote insects hitherto examined (*Campodea, Tomoerus, Lepisma, Anurida, Isotoma*) and even in *Eutermes* and some Orthoptera. Heymons’ work on the Orthoptera seems, in fact, to show the groove in process of evolution.

In 1886 Kowalewsky reinstated the insect gastrula, but in a modified form, and this has since been widely accepted. The ventral groove he regards as an elongate blastopore, the endoderm having become confined to its ends, with a long strip of mesoderm intervening. *Sagitta* is rather unconvincingly cited as analogy. Kowalewsky’s scheme derives support from the later work of Nusbaum and Fulinsky, Hirschler, Philiptschenko, and others, who describe in certain species the narrow median band of endoderm (Mittelstrang) connecting the anterior and posterior endoderm rudiments. Whether such a ‘gastrula’ conforms with Haeckel’s definition may perhaps be questioned; but the conception of endoderm arising by invagination at a blastopore has at least survived.

Even the extreme case described by Henson (1932) for *Pieris* might be reconciled with the theory. Here the mid-gut ‘Anlage’ arises, together with a small part of the mesoderm, from proliferation of the outer layer, but independently of the inner layer, the sites of proliferation occupying the place where stomodaeum and proctodaeum subsequently invaginate; since ‘ectoderm, mesoderm, and endoderm run indistinguishably into one another’ here, these regions may be the equivalent of a blastopore, with the divided blastopore of *Peripatus* as analogy.

But in *Calandra* with the mid-gut forming from stomodaeeum and proctodaeeum at relatively advanced stages of development (tracheae are already forming before the proctodaeal component arises) the limits imposed by the gastrula concept have been overstepped; it is impossible, even in principle to reconcile such an embryo with Haeckel’s notion of a gastrula, and it is obviously absurd to regard it as representing an extinct gastraea. As Nusbaum and Fulinsky (1909) have shown, we are able to construct a complete series connecting
the extreme cases described by Heymons with species which exhibit Kowalewsky's type of 'gastrula'; but it is evident that as this series progresses all trace of a gastrula is lost.

Earlier writers sought for the gastrula in the blastoderm stage of the embryo, the blastoderm-cells representing the ectoderm, the yolk-cells the endoderm. This view had at least the merit of identifying the gastrula at an early period of development, and not in comparatively advanced embryos as do the above discussed theories. It was based on the belief, then generally held, that the yolk-cells gave rise to the gastrula epithelium. With the discovery by Grassi, Kowalewsky, and others that the yolk-cells played no part in mid-gut formation this view was discarded in favour of Kowalewsky's scheme. Yet we have seen that it fails in Calandra.

Other writers (Will, 1888; Hirschler, 1912) even suggest a process of double gastrulation, adopting both the earlier and Kowalewsky's scheme; as Heymons comments, the insect would then recapitulate the gastrula ancestor twice in its own ontogeny.

Heymons himself inclined towards the earlier theory, seeking the gastrula in the blastoderm stage, the yolk-cells being abortive endoderm, while the mid-gut now arose from the ectoderm. Observations on Lepisma (1897a) and Campodea (1897b) appeared to confirm this view, for in those species the mid-gut was observed to develop from 'yolk-cells'. Yet judging by Uzel's (1898) account for Campodea there is no real comparison between such cells and the true yolk-cells of other insects, while in Scolopendra true yolk-cells and endoderm occur simultaneously (Heymons, 1901). Lepisma requires reinvestigation. Heymons' position depends on the propriety of homologizing yolk-cells with endoderm; as will appear below, there is no convincing reason for taking such a step.

We conclude then that a gastrula cannot be identified in the embryo of Calandra and probably of many other pterygote insects, without depriving that very useful concept of any meaning that may attach to it.

1 It should be observed that Balfour (1880), though he shared this view, justly refrained from identifying a gastrula in the insect embryo; thereby showing the misuse to which the term has been put by less critical writers.
With the question of the gastrula is bound up the thorny problem of identifying the germ-layers. It is not proposed to add yet another discussion to the many on this problem, for they can lead to no verifiable result; it will be more profitable to consider the bearing of the observations on the theory itself.

The indisputable fact of the existence of the germ-layers is the discovery of Pander; their theoretical interpretation is a later development.

In von Baer's great work of 1828-37 they are conceived simply as organs in the making—indeed he gives to the two primary layers the names 'dermal layer' and 'mucous layer', 'because they fully express the significance of these layers'. They are, for him, 'fundamental organs', and he is concerned but little with them until they have become the 'tubes' that form the early embryo. Their significance for him lies in the fact that they are general organs, from which the less general arise. With this conception of their nature the development of Calandra does not conflict.

But with the advent of the evolutionary interpretation of the germ-layers a change in meaning has crept in such that the development of a mid-gut from proctodaeum and stomodaeum, as in Calandra, seems to infringe some basic principle of development. Wherein does the change in meaning lie?

It seems to lie in the notion of the homology of the germ-layers, and in the application thereto of the principle of recapitulation. Haeckel, in giving expression to the doctrine of homologous germ-layers writes (loc. cit., p. 12): 'The metazoa form always two primary germ-layers...; their tissues always arise solely from the two primary layers, which have descended from the gastraea to all metazoa, from the simplest sponge to man.' Development is conceived as a process whereby these layers briefly recapitulate their ancestral history, and origin from a common embryonic rudiment becomes a criterion of homology; e.g. homology of the intestine of all metazoa is inferred from its universal origin from endoderm (loc. cit., p. 23). As a corollary, it will be observed, the notion of potentiality has now come in.

It is clear that 'endoderm' has become something more
fundamental than intestine. Haeckel, it is true, looked on the germ-layers as primitive organs (loc. cit., p. 258), for exceptions to the scheme were not contemplated; when however cases were discovered among insects where the intestine did not form from 'endoderm', the need seemed to arise for identifying this germ-layer in the embryo. Heymons (1895 a) then homologized the endoderm with the yolk-cells; Mansour (1927) with certain cells that were said to migrate early in development from the germ-band into the yolk and there degenerate. Reasons for rejecting both these interpretations have already been advanced; it will be more profitable to examine the principle involved.

Experimental methods have shown that development does not proceed in the manner contemplated by Haeckel and his contemporaries. A newt regenerates a normal crystalline lens, not from the epidermis but from its iris; a normal somite and mid-gut wall can arise from presumptive epidermis implanted into a gastrula (Mangold 1925). The course of development of a normal individual from a bud and from an egg are very different; while regeneration may even proceed in disregard of germ-layer specificity (Morgan, 1904). The ontogeny of a metazoan cannot, evidently, be conceived as a process whereby the two primary germ-layers recapitulate their ancestral history and unfold their potentialities. As Spemann (1915) writes: 'an organ is then no longer related through its 'Anlage' with the homologous organ of a nearer or more distant ancestor, but only indirectly, one might say only ideally, through the general potency of the germ to form the organ, and then its further ability to develop it at the homologous site.'

Homology is an inference from the study of organs. That it is applicable to embryonic organs must be conceded. But when applied to organ-forming regions of an embryo the concept becomes exceedingly vague, and it is doubtful whether any definable meaning then attaches to it. On this point Spemann (1915) writes: 'homologizing is only possible after the formation of 'Anlagen', i.e. at a developmental period when the individual parts of the germ have become differentiated, if not in their

1 For a criticism of Mansour's observation see section 7.
outward appearance, at least in their developmental tendency.’

Homology of an ‘Anlage’—in so far as it is an ‘Anlage’—is therefore determined by its fate rather than its origin. If, then, the gastral epithelium of all metazoa is homologous, so are also their ‘Anlagen’, whatever their mode of formation. But since the name ‘endoderm’ is applied to that component of the gastrula which is the gut ‘Anlage’, it is, in principle, not possible to homologize it with yolk.cells of an insect, when these do not actually give rise to the gut.

It is plain that the yolk.cells of insects are a specific embryonic organ, concerned probably with yolk.metabolism. If it is they that are homologous with the endoderm, i.e. mid.gut ‘Anlage’, of lower metazoa, then clearly the mid.gut ‘Anlage’ of insects cannot be; and the mid.gut of the adult insect is then also no longer homologous with that of other metazoa.1 Such are the difficulties which arise when we try to accommodate to the theory facts which were not contemplated in it; and a comparison drawn by Heymons (1901, p. 24) between the cleavage events of myriapods and certain annelids must, as a support for his thesis, now appear vague and unconvincing.

Although the mid.gut ‘Anlage’ in the embryo of pterygote insects is, surely, homologous with that of other metazoa, since the adult organs are, the term ‘endoderm’ is not applicable to it; for strictly this term must be reserved for the gut ‘Anlage’ only when this forms the inner layer of the gastrula.

To make the apparently ectodermal origin of the gut of

1 Lecaillon (1898) actually sought in this way to avoid conflict with the theory; and Mangold (1925) also suggests that the relationship is not one of pure homology (homogeny) but of homoplasy in Lankester’s (1870) sense. Yet similarity of development is not an essential condition of homogeny. Are not the normal and regenerated lenses of the newt’s eye ‘genetically related, in so far as they have a single representative in a common ancestor’, and therefore homogenetic? Lankester’s notion of homoplasy is apt to be misunderstood; indeed Spemann himself (loc. cit., p. 79) seems to commit this error when he regards the regenerated lens not as homogenetic but as homoplastic with the normal lens. For when Lankester speaks of ‘identical or nearly similar forces, or environments’, calling forth similar structures by acting on similar parts of one and the same organism, or on two different organisms, he uses the term ‘force’ in the evolutionary sense, and not in the physiological sense of ‘Entwicklungsmechanik’. 

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certain insects conform to theory the notion of 'latent endoderm' contained in the invaginating fore- and hind-guts, has been proposed. Heider offered this explanation in 1897, and a similar idea underlies the suggestion of Nusbaum and Fulinsky (1909); viz. that the development of the endoderm, which occurs at the site of stomodeum and proctodeum ingrowth, is delayed till after the formation of these structures, so giving to the mid-gut the appearance of arising from ectodermal organs. In so far as 'endoderm' is regarded as simply synonymous with 'mid-gut Anlage' this is, of course, true. But the germ-layer nomenclature is then retained at the expense of the theory. The essence of the germ-layer concept is the formation of two (or three) layers as a very early product of cleavage, the various organs then differentiating out of these layers. This is the sense in which Kowalewsky (1871) and Lankester (1873) and Haeckel (1877) use the term. Hertwig (1906), too, is very explicit on this point; by germ-layer formation 'is understood the initial organization of the embryonic cells into individual layers, from which, then, according to certain rules, all the organs and tissues take their origin'. It is evident that the notion of 'latent endoderm' is in direct conflict with the very essence of the theory, namely, the occurrence of visibly distinguishable layers of cells in the very early embryo.

We conclude, then, that 'endoderm' does not occur in the embryo of Calandra.¹ Haeckel homologized the gastral epithelium of metazoa on the ground of its universal origin from endoderm; it would seem, rather, that the organ-'Anlage' is primary, its occurrence as a germ-layer secondary. When the gastral epithelium arises very early, then endoderm forms; but when it does not appear till much later, there is no endoderm.

Recent advances in several branches of biology point to the need of a more general examination of the germ-layer theory; but this is quite beyond the scope of the present paper.

¹ The present observations must not be taken as a support for the paradoxical assertions that the midgut, in cases where it arises from stomodeum and proctodeum, is ectodermal; it is merely claimed that it cannot be endodermal.
10. LATER DIFFERENTIATION OF THE MESODERM, AND FORMATION OF THE EPINEURAL SINUS.

About the time the coelomic sacs are beginning to open a narrow ridge develops in the mid-line along the median unsegmented strip of mesoderm; it stretches from just behind the stomodaeum to about the level of the last thoracic segment, and in some embryos even into the abdomen. It arises by the local heaping up of mesoderm cells. Sometimes it projects very prominently, almost like a keel, into the yolk; at other times it is barely recognizable. It may be seen, though not well developed, in fig. 47, Pl. 23; a better example is shown in fig. 75, Pl. 24. Both in the character of its cells (they are rather spindle-shaped, with their axes longitudinal and do not show epithelial arrangement), and in its later development (it forms the blood-cells), it is to be distinguished from the more lateral parts of the median strip. These latter are to be reckoned as part of the ‘sub-somitic mesoderm’, and will be considered below; the median ridge is the ‘blood-cell lamella’.

It was first described by Nusbaum in Meloe, under the name ‘Chordastrang’. In German writings it is frequently referred to as the ‘Mittelstrang’, but as this name is also applied to the entire unsegmented median strip of mesoderm (cf. Hirschler, 1928) confusion will be avoided by using the term ‘blood-cell lamella’. A more non-committal name may, however, be required for this very definite structure, because in some insects (e.g. Phyllodromia—Nusbaum and Fulinsky, 1906) it is stated to participate in mid-gut formation. In Orthoptera and Dermaptera, according to Heymons, it forms exclusively blood-cells, and with this the present observations on Calandra agree.

While the coelomic sacs are forming, the nerve-cord is beginning to intrude as a median thickening into the interior of the embryo. The mesoderm between it and the coelomic sacs becomes greatly thickened. These thickenings are segmentally disposed; they fill the entire space between the nerve-cord and the coelomic sacs, and begin now to grow under the coelomic sacs themselves, from which, as yet, they are only indistinctly demarcated. Internally this ‘sub-somitic mesoderm’ (Eastham)
merges into the lateral parts of the median unsegmented strip, the term ‘sub-somitic’ being then conveniently applied to the whole. In those segments where appendages develop it merges into the mesoderm of the latter. This mesoderm of the appendage has meanwhile enlarged, and now forms a loose clump of cells completely obliterating its cavity. Reference to figs. 46 and 47, Pl. 23, will make this description clear.

In various insects—Gryllot alpa (Korotneff, 1885), Hydro philus (Heider, 1889), Donacia (Hirschler, 1909), the mature coelomic sacs are stated to exhibit definite openings into the epineural sinus. MacBride (1914) considers it likely that these openings are the result of the paraffin embedding to which the embryo has been subjected. In Wiesmann’s (1926) careful study of Carausius such openings were not seen. In Calandra they do not occur as such in the mature coelomic sac; later, however, when differentiation of the coelomic sac wall begins, with partial break-down of the wall, openings may appear, though usually these are occupied by a mass of mesodermal cells (fig. 75, Pl. 24).

Differentiation of the walls of the coelomic sacs begins early on the third day at a time when the germ-band is just beginning to shorten. From the splanchnic wall arises, as usual, the splanchnic musculature; the fat-body forms from the inferior part of the somatic wall, where it merges into the splanchnic; the external portion of the somatic wall gives rise to the lateral myoblast plate, while the vascular tissue is formed where the splanchnic and somatic walls meet dorso-laterally.

The first indication of differentiation is a loosening up of the inferior wall, the epithelial character of this part being now lost. A coelomic sac in the initial stage of this process is shown in fig. 74, Pl. 24. At a rather later phase of differentiation these cells are to be seen within the coelomic cavity, almost completely obliterating it; from them will develop the fat-body (fig. 75, Pl. 24).

All the coelomic sacs from the labial to the ninth abdominal undergo these changes. The antennary, of course, does not participate.

The further differentiation of the coelomic sacs is accom-
panied by much cell-division, and by enlargement of its parts. The cells of the fat-body begin to acquire their peculiar texture very early, and are soon distinguishable from neighbouring cells by their pale cytoplasm, by their exceptionally big vacuoles and consequently by their large size (figs. 76, 77, Pl. 24). The remainder of the wall of the coelomic sac has lost its epithelial character, the splanchnic muscle rudiment and the lateral myoblast plate being much enlarged and now well-defined. Although the 'Anlage' of the vascular tissue has now become distinguishable, actual cardioblasts cannot yet be recognized.

The subsomitic mesoderm, meantime, has much enlarged, and the fat-body soon begins to spread down over it.

The formation of the epineural sinus begins by a lateral withdrawal of those cells of the subsomitic mesoderm which immediately overlie the nerve-cord, these becoming incorporated into the main segmented masses. In the abdomen, late during the second day, a partial separation of the left and right halves of the mesoderm is seen, and, as the blood-cell lamella is absent here, a space is formed between nerve-cord and yolk. This is actually a precocious formation of epineural sinus (fig. 46, Pl. 23). In the thoracic and gnathal segments the sinus does not appear till well into the third day. Along its whole length the space thus formed expands to the width of the nerve-cord. Three stages in its formation are shown in figs. 76, 77, 78, Pl. 24, all representing sections through the first thoracic segment.

The common description of the epineural sinus arising by shrinkage of the yolk away from the embryo does not hold for Calandra; it is produced by a median withdrawal of mesodermal cells. Nor does it arise, as in Hydrophilus and Donacia as a paired space; it first appears in the mid-line.

11. ALIMENTARY CANAL (LATER DEVELOPMENT).

The development of the alimentary canal has, so far, been described to the stage where it comprises the following parts: (i) a short, blindly ending stomodaeum, which on the third day moves with the head on to the anterior pole of the egg, and so comes to lie horizontally; (ii) a proctodaeum, originally a dorso-ventrally compressed cleft, now become tubular, and, unlike
the stomodaeum, developing a wide opening on to the yolk; (iii) the mid-gut 'Anlage', in the form of two narrow but conspicuous bands of cells, adhering to the splanchnic mesoderm, merging behind into the ventral wall of the open proctodaeum, while anteriorly they converge, just behind the stomodaeum, on to the median sheet of cells from which a small amount of pre-oral mid-gut tissue grows forwards round the stomodaeum.

\[ \text{TEXT-FIG. 17.} \]

Advanced embryo, showing internal organs. \( \text{ao.}, \) aorta; \( \text{deuc.}, \) deutocerebrum; \( g., \) gonad; \( h., \) heart; \( \text{mal.}, \) malpighian tube; \( \text{m.flex.mand.}, \) flexor muscle of mandible; \( \text{m.g.}, \) mid-gut; \( \text{my.}, \) mycetocytes; \( \text{pr.}, \) proctodaeum; \( \text{prc.}, \) protocerebrum; \( \text{r.o.}, \) 'rectal organ'; \( \text{s.o.b.}, \) sub-oesophageal body; \( \text{s.o.g.}, \) sub-oesophageal ganglion; \( \text{st.}, \) stomodaeum; \( \text{Th.1.}, \) first thoracic ganglion; \( \text{trc.}, \) tritocerebrum.

\text{A. The Mid-gut.---With the movement of the head on to the front pole of the egg, during the third day, the yolk becomes pushed backwards. Owing to the development of the great head ganglia and the enlargement of the head muscles, the yolk, now much diminished in quantity, becomes pushed back well into the thorax (Text-fig. 17).} \\
\text{At the time the coelomic sacs are beginning to differentiate the associated mid-gut cells again start to multiply. They}
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spread at first downwards under the yolk; soon also, as the lateral body-walls enlarge, upwards, till on the fourth day the yolk becomes completely enclosed. That portion of the yolk which at an earlier stage lay pre-orally, now lies above the stomodaeum. The pre-oral mid-gut cells therefore form that portion of the intestinal wall which lies immediately above the stomodaeum; this will be understood from fig. 79, Pl. 24, where the cells still form a little clump, and have not yet spread as an epithelium over the yolk.

Throughout this period the mid-gut cells are characterized by their hyaline and slightly vacuolated cytoplasm. At the time the spreading begins they are usually packed several cells deep; gradually, however, they separate to form the simple epithelium of the larva. This is accompanied by a large increase in their volume. Scattered in considerable numbers among these large cells are smaller ones, with nuclei less than half the diameter of those of the functional mid-gut cells; they are the 'replacing cells' whose further development is described in our previous paper (Murray and Tiegs, 1935). They are to be seen in fig. 71, Pl. 24, and are already recognizable in the section drawn in fig. 78, Pl. 24.

As the mid-gut cells gradually spread round the yolk the associated layer of splanchnic mesoderm, in which all trace of segmentation is now lost, spreads with them, and so ensheaths the mid-gut epithelium. A clear line of demarcation is always visible between the two layers. Segments anterior to the labial do not contribute splanchnic mesoderm to the intestinal wall, the mesodermal sheath investing its anterior end being derived from cells that move forwards from the labial segment. This does not, actually, involve any extensive migration of cells, for it is at this period that the gnathal segments are becoming reduced in size and only the most anterior tip of the mid-gut lies before the labial segment.

Although most of the splanchnic wall of the mid-gut thus comes from the coelomic sacs, an exception must be made for a portion which lies just dorsally to its connexion with the proctodaeum. If fig. 66, Pl. 23, be examined a thin sheath of cells will be seen closely investing the yolk. These cells are
derived from the mesoderm that has migrated forwards from the eleventh segment, but most of which is used in the formation of the investing sheaths of the proctodaeum and malpighian tubes (see below 11 C). With the completion of shortening of the germ-band the position of this membrane, which has till now faced downwards, becomes inverted. But owing to withdrawal of yolk from the hinder end of the embryo the membrane becomes drawn forwards with it, and so forms the splanchnic sheath for the postero-dorsal portion of the mid-gut. Reference to Text-fig. 17 will make this description clear.

Differentiation of the splanchnic coat into circular and longitudinal muscle layers, and probably also into serosa, occurs during the fourth day.

During the last day the mid-gut assumes its definitive form. At about the end of the third day, when the embryo has just completed its shortening, the yolk still extends backwards, dorsally, well towards the hinder end of the embryo, though ventrally it is displaced forwards by the enlarging proctodaeum (cf. the more advanced embryo in Text-fig. 17). But after enclosure of the yolk has been completed it undergoes a rapid absorption. The mid-gut at the same time alters its form, being converted from a large ungainly sac into a more elongate but still spacious organ. In front it is wide; the hinder end, however, particularly where it merges into the proctodaeum, has become quite narrow. The characteristic coiling, previously described (Murray and Tiegs, 1935) appears during these events.

The intestinal caeca, which are confined to the narrow hinder portion of the mid-gut arise, in the latter half of the fourth day, as solid outgrowths from the mid-gut epithelium (fig. 71, Pl. 24); the intercellular lumen forms later.

B. The Stomodeum.—By the end of the second day the stomodeum has become cylindrical with a narrow lumen, and projects prominently into the yolk.

At about this period cells begin to separate off from the blind end of the stomodeum and migrate in a long string into the yolk (fig. 64, Pl. 28). They have already been referred to by Mansour (1927). Their fate is described below (11 E).
With the beginning of shortening of the embryo the enlarging head, as already described, moves on to the anterior pole of the egg. This brings the stomodaeum into a horizontal position (fig. 79, Pl. 24). The yolk, at the same time, becomes pressed backwards, as the great head ganglia develop. This is attended by a great elongation of the stomodaeum, due to intense proliferation of its cells. In some embryos it even bulges deeply into the yolk.

Active proliferation of its cells continues till about the end of the third day, the stomodaeum being now a thick-walled blindly ending tube, composed of densely packed cylindrical cells. The further enlargement which occurs in the last day is due, not to multiplication of cells, for mitosis is not seen, but to a change in the form of the cells, which become cubical or even flattened. The hind end of the stomodaeum at the same time expands to form the crop.

During the fourth day the tip of the stomodaeum becomes reduced to an exceedingly thin membrane. Its condition at about the end of the third day is shown in fig. 79, Pl. 24. Many of its cells now undergo transformation into mycetocytes (fig. 101, Pl. 25) which then become incorporated into the main mass of mycetocytes which is accumulating round the stomodaeum at this period (v. below 11 E). Only a few cells remain behind to form a partition between the yolk and the lumen of the stomodaeum. As the end of the stomodaeum expands to form the crop this membrane becomes stretched to an almost imperceptible fineness. It persists thus up to about the time of emergence.

The muscle-coat of the stomodaeum is derived chiefly from the pre-oral mesoderm, though the post-oral (i.e. premandibular) also contributes a part. In Pieris Eastham (1930 a) finds that the pre-oral mesoderm alone is concerned; but Forficula, according to Heymons' account, resembles Calandra. The two masses of mesoderm appear in the section shown in fig. 79, Pl. 24, the pre-oral being much the more conspicuous. In fig. 85, Pl. 25, which represents a horizontal section along an embryo at the beginning of shortening, the origin of this mesoderm from the premandibular segment is seen; reference to Text-fig. 12...
will make the relationship clear. As development progresses, the two masses form a complete investment for the stomodaeum (fig. 89, Pl. 25). Differentiation into circular and longitudinal muscle-coats occurs during the fourth day; the small oesophagaeal dilators (figs. 116, 118, Pl. 26) also arise from it.

C. The Proctodaeum.—In the foregoing account the proctodaeum has attained the condition of a short tubular organ, opening internally on to the yolk, its thick ventral wall being continuous with the mid-gut 'Anlage' that has arisen as a pair of band-like outgrowths from it.

The internal opening of the proctodaeum survives till early in the third day. Closure begins by the formation, immediately in front of the opening of the malpighian tubes, of a transverse partition derived entirely from proctodaeal cells. By the time the germ-band has completed its shortening a second layer has been formed, from the mid-gut epithelium.

Meanwhile, the proctodaeum elongates rapidly owing to much division of its cells. During this phase of rapid growth its wall is a thick epithelium of narrow columnar cells. Differentiation is first seen early on the fourth day. The intense cell-division has now ceased; the cells begin to enlarge, particularly along the floor of the hinder (rectal) portion, while at the same time the organ as a whole elongates and begins, in consequence, its characteristic coiling (Text-fig. 17). The differentiation continues during the fourth day.

Communication between mid-gut and hind-gut is re-established late on the fourth day. In fig. 73, Pl. 24, is shown a sagittal section of the intestine at the junction of the two, immediately prior to break-down of the partition. From the appearance of the cells it seems probable that they become withdrawn into the intestinal wall.

Both the musculature of the hind-gut, and the peculiar organ referred to as 'rectal-organ' (Text-fig. 17) in our previous paper (Murray and Tiegs, 1935) are derived from the large mass of mesoderm cells situated behind the genital rudiment. As the proctodaeum becomes tubular and elongates these cells move forwards, and envelop it along its whole length. Into this mass before it has yet become organized into an investing sheath
for the proctodaeum, the three pairs of malpighian tubes grow.

Some of the mesodermal cells now show a tendency to apply themselves to the walls of the latter (fig. 67 A, Pl. 24), others form a sheath for the proctodaeum itself. With the formation of the haemocoelic this portion of the embryo becomes 'loosened up'. We can then clearly distinguish the following (fig. 66, Pl. 24):

(i) a thick mesoderm sheath investing the rectum, and giving rise to the rectal muscle-coats, the 'rectal-organ' and the investing serosa; (ii) a thin sheath ('serosa') investing each malpighian tube; (iii) the thin sheath, alluded to above (section 11 A), from which will arise part of the splanchnic coat of the mid-gut.

Differentiation of the 'rectal-organ' occurs surprisingly early, namely at the time when the germ-band has just completed its shortening. The rectal musculature does not differentiate till the end of the fourth day.

D. The Yolk.—Division of the yolk-cells, described in section 3 B, does not occur beyond the blastoderm period. With the formation of the germ-band the nests of yolk-cells give place to single cells scattered, as a syncytium, through the yolk. By about the middle of the second day the syncytium is beginning to break up into parts, till eventually each nucleus becomes the centre of a small yolk-sphere. At first multinuclear masses occur (figs. 51, 52, 55, Pl. 22), but at about the end of the second day have usually become resolved into single cells (fig. 64, Pl. 22).

During the third day, when the yolk is being rapidly absorbed, many of these yolk-nuclei are seen in stages of degeneration or disintegration. Thereafter the yolk gradually becomes a disorganized mass of yolk- and fat-globules, nuclei, and fragments of cytoplasm with only seldom a sign of intact cells (figs. 79, Pl. 24; fig. 115, Pl. 26). Remains of the yolk survive till after emergence of the larva.

E. The Symbiotic (?) Bacteria and the Mycetoma.—The peculiar relation that exists between a bacterial organism and certain tissues of Calandra oryzae has been recorded in several recent papers (Pierantoni, 1927; Buchner, 1930; Mansour, 1930; Murray and Tiegs, 1935). The manner
of infection of the sexual organs is described in section 4; here we describe the relationship to the gut.

At the end of the second day isolated clumps of bacteria are occasionally encountered in the yolk (fig. 64, Pl. 23). To these now become added great masses of bacteria that arise from the disruption of mycetocytes that migrate into the yolk from various parts of the developing gut-wall.

The first to appear are a group of cells which become liberated from the tip of the stomodaeeum, and migrate as a long string of cells into the yolk (fig. 64, Pl. 23). At the time of their first appearance they are not visibly distinguishable from the adjacent stomodaeal cells. But in more advanced embryos, when the germ-band is beginning to shorten, they have completely changed their appearance, and are now seen to be packed with a felt-work of bacteria. Many of these mycetocytes disrupt, great clumps of bacteria with nuclei scattered among them thus coming to lie freely in the yolk.

To this bacterial mass are now added cells, already observed by Mansour (1927), which migrate from the walls of the developing mid-gut. From the mid-gut 'Anlage' as it spreads upwards over the yolk, and particularly from its dorsal edge, sheets of cells spread inwards into the yolk (fig. 77, Pl. 24). In appearance they are indistinguishable from normal mid-gut cells. Within the yolk they enlarge and become converted into mycetocytes (figs. 77, Pl. 24; fig. 115, Pl. 26). Many remain intact; but mostly they disrupt, shedding their bacterial content into the yolk, where it forms conspicuous masses of a dense feltwork of bacteria, lying amidst the yolk.

Formation of the mycetoma begins by an accumulation of the intact mycetocytes round the blind end of the stomodaeeum (fig. 79, Pl. 24; Text-fig. 17). As this mass enlarges, mycetocytes, that arise by transformation of cells from the blind end of the stomodaeeum, become added to it; indeed, most of the cells of the immediately adjacent stomodaeeal wall seem to undergo this fate (fig. 101, Pl. 25). Eventually, as Mansour observed, the whole forces its way out from the yolk and becomes lodged as the conspicuous mycetoma just below the crop (figs. 69, 70, Pl. 24; Text-fig. 18).
A review of the literature on the adaptation of embryonic events to symbiosis is beyond the scope of this paper. Buchner's comprehensive work (1930) may be referred to. Nothing comparable with the remarkable events observed in Calandra oryzae seems to have been hitherto recorded.

12. The Salivary Glands.

These are associated not with the labium but with the base of the maxillae, on the inner aspect of which they open. They arise late on the third day as long tubular invaginations of the ectoderm, and extend back as far as the mycetoma, where they coil a little. Fig. 79, Pl. 24, shows an early stage of development.

13. The Malpighian Tubes.

In the larva three pairs of malpighian tubes occur. They open into the anterior end of the hind-gut, and doubling back on themselves, are attached at their blind ends to the rectum. One pair is short; the other two much longer, one of these being particularly long and extending to the anterior end of the mid-gut before bending back (for illustration see Murray and Tiegs (1935), Text-fig. 1).

One pair much precedes the other two in time of development. The first two tubes arise in the latter half of the second day as a pair of invaginations from the floor of the proctodaeum (fig. 62, Pl. 23). In some exceptional cases they are distinguishable at a time when the hinder end of the germ-band is just bending down into the yolk (fig. 60, Pl. 28); but usually they appear rather later (fig. 61, Pl. 23). Although they arise so early their further development is retarded till about the end of the second day when they begin to elongate. They grow backwards alongside the proctodaeum, and acquire an investing sheath of mesoderm in the manner already described (section 11 C).

No indication of the remaining malpighian tubes has been seen in any embryo till early in the third day, at a time when the proctodaeum has become converted into a cylindrical tube. As the tubular form develops the openings of the first pair of malpighian tubes are carried on to the sides of the proctodaeum (fig. 67 b, Pl. 24). The remaining tubes arise, like the first, as
hollow outgrowths of the proctodaeal wall, a little behind and dorsal to the first pair (fig. 67 A, Pl. 24). Like the first they grow backwards alongside the proctodaeum, so that three pairs appear simultaneously in transverse sections through this region, of which the first pair is distinguished by its greater size (fig. 66, Pl. 23).

During the third day active cell-division causes much elongation of the tubes. Late on the third day the proctodaeum begins to bend, and with it the malpighian tubes (Text-fig. 17). A phase of differentiation, unaccompanied by cell-division, sets in, and the cells, hitherto rather columnar and loosely packed, become considerably flattened, while the whole tube becomes thinner and develops a more compact texture, the duct itself becoming better defined. Simultaneously the tubes lengthen, particularly the two larger pairs; the blind hinder end retains connexion with the wall of the proctodaeum, while the middle portion is pressed forwards in the haemocoele, and so adopts the peculiar form seen in the larva.

Already at this early period two kinds of cells—with small and with large nuclei—are distinguishable in the wall of the malpighian tube (fig. 72, Pl. 24); it is very probable that they are the imaginal and larval cells respectively. The ultimate enormous disproportion in their size appears only later during the growth of the larva (see Murray and Tiegs, 1935).

14. THE SUB-OESOPHAGEAL BODIES.¹

The organs referred to by this name were first described by Wheeler in 1893 from the embryo of Xiphidium and have since been seen in a variety of species. They are stated to be more conspicuous in the embryo than in the larva, and do not survive into the imago. In this latter respect Calandra is an exception.

¹ The occurrence of this organ in the larva was overlooked in our previous paper. It is found throughout the larval period as a small paired flattened cell-mass, adhering to the underside, and in fact, incorporated into the body of, the mycetoma. Here its cells grow markedly in size. It survives the metamorphosis, occurring in the imago as a pair of inconspicuous bodies at the hinder end of the gizzard. The number of its cells has become reduced to about 9–12. They are clumped into several masses in which cell-boundaries are hardly recognizable. Their appearance suggests nephrocytes.
Embryologically the bodies are of interest because two distinct methods of development have been assigned to them. They are generally held to be derivatives of the premandibular (intercalary) mesoderm—Orthoptera (Wheeler, Heymons, Wiesmann); Isoptera (Strindberg), Pieris (Eastham), Wiesmann stating that in Carausius they arise quite obviously from the premandibular somite. Yet according to Nusbaum and Fulinsky (Orthoptera), Hirschler (Donacia), Schwangart, Hirschler, and Johannsen (various Lepidoptera) they are mid-gut derivatives. Whether there is uniformity in their development must, for the time, remain uncertain; present observations on Calandra, however, offer a possible explanation for this difference of opinion, for though the bodies arise from the mesoderm they become secondarily part of the mid-gut wall and have the appearance of arising from the latter.

They first become clearly recognizable in embryos in which shortening is just beginning, appearing as a pair of rounded bodies, often enclosing a cavity, and located at the anterior angle of the mandible just behind the stomodaeum, i.e. in a region corresponding to the intercalary segment. This is readily seen in the horizontal section shown in fig. 85, Pl. 25. Externally they abut on the tritocerebral ganglion; internally they are in contact with the yolk (fig. 84, Pl. 25). Although a few mid-gut cells already appear in association with them, they are not actually a part of a definite mid-gut wall; in later embryos, however, when a complete mid-gut epithelium has become established, they appear as part of the mid-gut wall, being situated as a pair of very conspicuous bodies immediately ventro-lateral to the base of the oesophagus (fig. 79, Pl. 24), and giving the appearance of having developed as outgrowths from the mid-gut. The cells throughout this period are already distinguishable by their large size, and by the paleness and characteristic faint granulation of their cytoplasm.

It remains then to determine whether they are derivatives of the mid-gut cells, or whether they come from the mesoderm. The latter proves to be the case. This is well seen in fig. 86,
Pl. 25, which represents part of a section of a transversely cut, rather earlier embryo, taken at about the same level as that shown in fig. 84, Pl. 25, i.e. through the tritocerebral ganglion. It will be seen that the cells of the sub-oesophageal body, already distinguishable by their size and peculiar texture, occupy the position of mesoderm cells. A definite somite, as described by Wiesmann for Carausius, they do not form; yet at a rather later phase, as shown in figs. 84, 85, Pl. 25, they show often a remarkable resemblance to coelomic sacs.

In criticism of Hirschler's (1907, 1928) contention that the sub-oesophageal body arises from the mid-gut and not from the intercalary mesoderm, it should be observed that, according to his observations on Donacia, this mesoderm, in the form of a pair of large coelomic sacs, is utilized in the formation of the cephalic aorta; yet general experience shows the aorta to arise from the antennary coelom (which is said to be absent in Donacia), or at any rate, from antennary mesoderm, while an intercalary coelom occurs only in the most primitive insects, and then as a vestige at most. It seems very probable that the Donacia embryo has been misinterpreted; an examination of figs. 83, 84, 85, and 87, Pl. 25, will show how easily antennary and intercalary mesoderm can be confused.

In later embryos of Calandra the sub-oesophageal bodies again lose connexion with the mid-gut. This occurs during the fourth day. As the mycetocytes begin to move through the gut-wall they carry the sub-oesophageal bodies before them. The cells of the latter begin to spread out on the ventro-lateral parts of the mycetoma, into the contours of which they become incorporated (figs. 69, 70, Pl. 24). Histologically the two tissues remain distinguishable.

A formation of blood-cells from the sub-oesophageal bodies, as described for some species, does not occur in Calandra.

15. The Corpora Allata.¹

According to all who have investigated the matter, these bodies are ectodermal. Heymons (1895) in Forficula was the

¹ These organs were not referred to in our previous account. In the newly hatched larva they occur as a pair of compact ovoidal bodies, with radial
first to describe their formation; from his account they appear to arise as ingrowths from the anterior angle of the maxilla, a pair of rounded bodies becoming constricted off and eventually finding their way, by the aid of the maxillary tentorium, to the antennary coelomic sacs, to the lower ends of which they then attach themselves. This appears to be the case also in Bacillus rossii (Heymons, 1897 c), Chalicodoma (Carrière and Bürger, 1898), Formica (Strindberg, 1913), Apis (Nelson, 1915), and Pieris (Eastham, 1930 a).

There seems to be no reason for doubting these accounts; yet in Calandra the bodies develop quite differently. They arise from the antennary segment, and though they form in intimate association with the tentorium, they are, as far as could be ascertained, purely a differentiation of the ventral wall of the antennary coelomic sac, i.e. they are mesodermal.

By the beginning of the third day, when the germ-band has started to shorten, the antennary coelomic sac (section 8) has become clearly defined as a small vesicle which may project a little into the cavity of the antenna, though the major part of cell arrangement, at the anterior ventral surface of the brain, a little behind the circum-oesophageal nerve-strands. To the brain they are joined by a large tracheal vessel that enters the latter; with the cephalic aorta they are connected by membrane (remnant of the antennary coelomic sac), which itself forms a delicate investment for the corpora. Their position is shown in Text-fig. 18. A nervous connexion with the sympathetic could not be detected.

During larval life the bodies enlarge considerably but remain in position under the brain. But already in the early pupa they have become drawn up to their definitive position in the dorso-lateral wall of the oesophagus, to the side of and just behind, the hypocerebral ganglion. The tracheal connexion with the brain is still present. Nabert (1913) has already described a similar movement of the corpora allata during metamorphosis in a number of species.

In the young larva they form a small solid ball of cells with deeply staining peripheral nuclei. During the larval period the cells become about doubled in number and also enlarge. The middle of the organ presents a granular eosinophile protoplasm (ground substance of gland); cell-walls are recognizable only at the outer nucleus-bearing periphery. At the onset of metamorphosis the ground substance often presents a peculiar hyaline appearance, and may exhibit nuclei. In aged adults (5-6 weeks old) the bodies appear markedly swollen, and the nuclei much enlarged.
it lies behind the appendage (figs. 83, 86, 87, Pl. 25). Two events now occur which bring about a backward movement of the coelomic sac—the development of the brain and of the tentorium. As the brain gradually develops by thickening of the ectoderm in front of the antenna and as it enlarges and grows backwards, it pushes the underlying antennary coelomic sac far back into the cavity of the head. The development of the tentorium (q.v.) keeps pace with these events. We are concerned here only with the antennary component of the tentorium. This grows back as a tubular ingrowth of the ectoderm from just behind the antenna, to meet eventually a similar ingrowth from the hinder angle of the maxilla. As the ingrowth from the antenna develops, a loose connexion is observed between it and the adjacent coelomic sac, due to the outgrowth of fine protoplasmic strands from cells in the ventral wall of the latter. Fig. 79, Pl. 24 and fig. 88, Pl. 25, will make this description clearer. They are drawn from longitudinal sections of a single embryo, in which the plane of section is tilted considerably from the sagittal plane. In fig. 79, Pl. 24, only the lower part of the coelomic sac appears, but its relation to the sub-oesophageal body (i.e. premandibular mesoderm) is shown. In fig. 88, Pl. 25, from the opposite side of the same embryo the connexion with the tentorium is seen, as well as its primitive relation to the brain.

In the meantime the coelomic sacs have considerably enlarged, mitosis of their cells being frequently seen. The cells are distinguishable from those of the adjacent tentorium by the rather deeper staining of their nuclei.

Formation of the corpora allata begins with a thickening of the ventral wall of the coelomic sac (figs. 88, 89, Pl. 25). The cells of the thickening present the distinguishing features of the remaining cells of the sac wall, there being no evidence for a migration of ectodermal cells of the tentorium over to it. The section shown in fig. 89, Pl. 25, it should be explained, passes just behind the antenna, the posterior wall of the latter being partially included in the section; the large mass of cells between it and the transected tentorium being also tentorial cells cut along their line of ingrowth from the base of the antenna.

A later stage of development is shown in fig. 90, Pl. 25.
Only a portion of the coelomic sac, which is now much enlarged, is shown in the drawing. Its walls have become thinner, and the corpus allatum sharply defined.

A rather later stage is shown in fig. 105, Pl. 25 and fig. 116, Pl. 26. While the coelomic sac has greatly elongated and thinned out, the corpus allatum has become more distinct still, while the tracheal vessel, referred to in the appended footnote, p. 238, has come into association with it.

In fig. 91, Pl. 25, from an embryo in which shortening is not quite complete, the body has become invested in a sheath; and although a connexion of the coelomic sac with the tentorium survives, the body itself is no longer connected with the tentorium.

Finally during the fourth day the cells differentiate; they enlarge, and adopt a radial arrangement, their cytoplasm becoming more eosinophil, and in this way occur in the newly emerged larva.

Since these conclusions differ radically from those of all other workers, it is desirable to make some comments on their objective validity. An origin from the base of the maxilla is excluded by the facts (i) that the body may be observed in process of differentiation from the walls of the coelomic sac, (ii) that in some embryos the body is in an advanced state of development before the tentorial ingrowth from the antenna has met that from the maxilla, the participation of the latter in transferring the corpora allata to the coelomic sacs, as described by Heymons for Porificula being thereby excluded. The chief trouble in the present work has been the possibility of migration of cells on to the coelomic sac from the adjacent antennary ingrowth, which would render them ectodermal. In the absence of marking experiments reliance has been placed only on the visible, though often not very well defined distinction in the appearance of the two types of cell; throughout their development the cells of the corpora allata resemble those of the coelomic sac and not of the tentorium.


A. The Haemocoel.—This arises late on the third day in embryos in which shortening has begun, the first evidence of
its formation being the appearance of the epineural sinus. Its development is described in section 10.

At about the end of the third day the fat-body, now much enlarged, separates from the adjacent lateral walls of the mid-gut, the lateral blood sinus, merging below into the epineural sinus, thus arising (fig. 78, Pl. 24; fig. 115, Pl. 26). When the lateral sinuses eventually merge into one another above the gut, the latter becomes entirely contained within that space.

In the meantime, by the separation of the nerve-cord from the epidermis, the latter also becomes contained within the haemocoel (figs. 77, 78, Pl. 24; fig. 115, Pl. 26).

By separation of the proctodaeum from the adjacent fat-body the haemocoel spreads into the terminal body segments (fig. 66, Pl. 23).

In the head also a prominent space appears. It arises after shortening of the embryo has begun, and owes its formation to a withdrawal of the yolk from the head region, due to its gradual absorption and its enclosure within the mid-gut. The space so formed, which is continuous behind with the haemocoel of the thorax, is partly occupied by the enlarging antennary coelom (fig. 89, Pl. 25); but with the complete withdrawal of the yolk, and with the subsequent diminution in size of the antennary coelom, it becomes greatly enlarged and accommodates the brain (figs. 105, Pl. 25; fig. 116, Pl. 26).

B. The Blood.—The blood-cells are derived entirely from the ‘blood-cell lamella’—the median unsegmented ridge of mesoderm cells that extends from just behind the stomodaem to the level of the last thoracic, or even, occasionally, second or third abdominal segment (section 10).

At the time the embryo is beginning to shorten, these cells, hitherto spindle-shaped and exhibiting marked longitudinal polarity, become rounded, and by the time the epineural sinus has formed, have acquired the peculiar histological features—clear hyaline and often slightly vacuolated cytoplasm—by which they can usually be identified (cf. fig. 77, Pl. 24). Although in *Calandra* they arise only in the anterior half of the embryo they may, late in the third day, be encountered floating at random anywhere in the epineural sinus.
No other source of blood-cells, whether from the heart or from the sub-oesophageal body, could be detected.

According to Nusbaum and Fulinsky (Phyllodromia), Hirscher (Donacia), and Philipschenko (Isotoma) mid-gut cells arise from the same primitive 'Mittelstrang' which is the source of the blood-cells; there is, however, no evidence for this in Calandra, in which respect the present observations agree with those of Korotneff (Gryllotalpa), Heymons (Orthoptera and Dermaptera), and Eastham (Pieris).

C. The Fat-body.—This occurs, in Calandra, in two distinct parts: (i) a bulky visceral portion occupying most of the haemocoele, and in later larvae almost obliterating it; (ii) a comparatively inconspicuous parietal zone of smaller less vacuolated cells, lying just under the epidermis and external to the muscles (Murray and Tiegs, 1935).

The main portion of the fat-body is derived from the inferior wall of the coelomic sacs from the labial to the ninth abdominal segment. The early stages are described in section 10. After separating from the wall of the coelomic sac the cells lose their capacity for deep staining and become more and more highly vacuolated. The fat-cells thus rapidly enlarge, and spread towards, but not into the epineural sinus, while laterally the body extends upwards in the haemocoele as the walls of the embryo encircle the yolk. Although fat-cells arise in the labial segment they do not become included in the head. In advanced embryos the fat-cells clump together into multinucleated masses.

The parietal fat-body arises independently of the visceral, and seems to be derived from cells originating in the external wall of the coelomic sac. At about the end of the third day, when the development of the visceral fat-body is already advanced, a layer of rather large scattered cells is seen between the epidermis and the lateral masses of myoblasts. They do not appear to arise from the adjacent ectoderm, but seem rather to be small remnants of the cells of the external coelomic sac-wall, that have not been utilized in muscle formation. A few parietal fat-cells are seen in figs. 112, 115, Pl. 26.

D. The Dorsal Blood-vessel.—This comprises (i) the heart proper, with four ostia and four main alary muscles,
(ii) the aorta, (iii) the cephalic aorta. The heart and aorta will be considered first.

These develop from the dorso-lateral walls of the coelomic sacs. Every such sac, from the ninth abdominal forwards to the labial, is concerned; but as far as could be determined the gnathal mesoderm anterior to the labial does not contribute to their formation.

Within the intact coelomic sac it is impossible to identify the limits of the cardiac tissue for, unlike some insects, not even the cardioblasts are distinguishable till much later; in Donacia Hirschler could detect cardioblasts in the mature coelomic sac, while in Forficula Heymons observed a conspicuous para-cardial-cell ‘Anlage’.

By the time the embryo has begun to shorten, concrescence of the coelomic sacs is well advanced. The cardiac ‘Anlage’ now becomes stretched to a thin membrane between the splanchnic mesoderm and the ectoderm, abutting on the yolk above, and covering the fat-cells below (fig. 76, Pl. 24), sections taken along the lateral body-wall revealing it as a columnar epithelium in which traces of the initial segmentation have still survived (fig. 108, Pl. 25).

Cardioblasts first become distinguishable in the anterior segments, appearing as a succession of enlarged cells immediately adjacent to the ectoderm (fig. 77, Pl. 24, from an embryo at about the stage of Text fig. 18).

In the abdomen the development of the alary muscles and the nephrocytes (paracardial tissue) introduces a complexity. A section through the ‘Anlage’ at the level of the first alary muscle, just before differentiation of the cardioblasts, is shown in fig. 108, Pl. 25. The conspicuous mass of mesoderm adjacent to the ectoderm is only partly concerned with heart formation, some of its cells being somatic myoblasts. An early stage in its differentiation into cardioblasts, alary muscle, and myoblasts is seen in fig. 109, Pl. 25. A more advanced stage, from an embryo that has nearly completed its shortening, is seen in fig. 110, Pl. 25; cardioblast, alary muscle, pericardial, and paracardial rudiments are now all apparent.

With the spreading of the lateral body-wall upwards over the
sides of the yolk the two heart rudiments (Anlagen) are carried on to the dorsal surface of the embryo and there meet. Closure occurs early on the fourth day, appearing first at the anterior, later at the posterior end, and most delayed in the mid-region.

The formation of a closed tube is attended by a change in shape of the cardioblasts. Till now the cardioblasts on each side have formed a row of very large flattened cells placed, like a row of books, with their flat surfaces apposed, so that in lateral view they give a peculiar pallisade appearance (fig. 113, Pl. 26) while the true width of the cell is visible only in transverse section (fig. 111, Pl. 26). But as the tube develops they change into long curved cells (fig. 112, Pl. 26).

The ostia are recognizable as clefts between the cardioblasts (fig. 118, Pl. 26).

Throughout this period of development the heart tissue remains loosely connected by a ‘mesentery’ with the splanchnic mesoderm that invests the mid-gut. This is best seen at the hinder end of the embryo where the gut shrinks well away from the body-wall. In Hirschler’s description for Donacia the cavity of the heart, incompletely closed below, is said to be temporarily continuous through the ‘mesentery’ with a narrow sinus surrounding the proctodaeum, and in Carausius Wiesmann found a well-developed sinus around the mid-gut. There are indications in Calandra also of such a sinus at the posterior end of the mid-gut, but it is, at best, only a narrow ill-defined cleft (fig. 111, Pl. 26). In later embryos the last remnant of ‘mesentery’ disappears (fig. 112, Pl. 26).

Differentiation of the various associated cells occurs very late. In fig. 114, Pl. 26, from an advanced embryo the network of pericardial (adventitial) cells, as well as the clumps of nephrocytes is seen; it will be observed that the latter have remained segmental.

Blood lacunae such as occur in various Orthoptera (Korotneff, 1885; Heymons, 1895) prior to formation of the heart, are not present in Calandra.

The cephalic aorta is formed from the antennary coelomic sacs. The earlier development of these sacs is described in section 15.
While the corpora allata are differentiating from their lower ends the walls of the sacs are becoming thinner and thinner, the small compact vesicles becoming converted into long delicate sacs which extend to the side of the stomodaeum underneath the protocerebrum up towards the most anterior cardioblasts.

In backwardly sloping, rather than transverse, sections it is possible to include the coelomic sac for its whole length within a single section. Fig. 105, Pl. 25, is drawn from such a section; at its lower end the coelomic sac, now very attenuated, is attached to the tentorium, and with it is connected the corpus allatum, while at its upper end it has come into association with the most anterior cardioblasts of the same side.

A later stage of development is shown in fig. 106, Pl. 25. The two rows of cardioblasts have united to form a tube, here cut longitudinally, for it is bending down over the anterior end of the mid-gut (cf. Text-fig. 18); the two coelomic sacs have become intimately connected with the end of the aorta, and it will be observed that while their internal walls are continuous with the aorta itself, their external walls fuse with the adventitia. In the more advanced stage shown in fig. 107, Pl. 25, the internal wall of the coelomic sac has assumed the structure of the wall of the aorta.

It will be evident, and this can be confirmed in appropriately cut sections, that the cephalic aorta has arisen by the union of two grooves on the internal dorsal faces of the sacs. The aorta thus formed passes above the ventricular ganglion; but at the level of the hypocerebral ganglion closure occurs in such a way that the ganglion itself becomes enveloped by it (Text-fig. 18).

The anterior opening of the cephalic aorta is a little in front of the hypocerebral ganglion. The adventitial layer, in addition to forming the very delicate investment of the aorta, also spreads downwards in this region as a thin membranous hood, attached below on either side to the tentorium, and having the corpora allata still appended to it. In addition to attachment to the tentorium it is also supported by a long and exceedingly fine filament which is inserted in front on to the head capsule. The whole organ is shown in Text-fig. 18. It will be evident, in comparison with fig. 105, Pl. 25; fig. 116, Pl. 26,
that it is the ventral part of the coelomic sac, the aorta forming from only the dorsal portion.

The origin of the cephalic aorta from the antennary coelom accords with general experience (Forficula Heymons; Eutermes and Formica Strindberg; Apis Nelson; Carausius Wiesmann); Hirschler's contention that it arises from an intercalary coelom sac is discussed in section 14.

17. The Epidermis and Simple Derivatives.

A. The Epidermis.—To the end of the third day this is a closely packed columnar epithelium. After complete enclosure
of the yolk differentiation begins, the cells becoming cubical and chitinizing on their outer face. There is no evidence for a special embryonic cuticle.

The structure of the thoracic appendages of the embryo is shown in figs. 76, 77, 78, Pl. 24; the same appendage (first thoracic) from a newly hatched larva is shown in fig. 102, Pl. 25. It has become withdrawn to the level of the surrounding epidermis, and survives as the imaginal disk of the leg, and is recognizable only as a rather deeply staining thickening of the epidermis.

The structure of the mouth-appendages is quite different, for they are not hollow, but consist of a solid mass of long filamentous epidermal cells, the nuclei congregating towards the base of the appendage.

B. The Oenocytes.—These are confined to the abdomen and occur as clusters of cells situated in the fat-body just behind the stigmatic trunks.

They arise, as in other insects, from the epidermis. After the embryo has begun to shorten they become recognizable as small clumps of enlarged cells in the epidermis just behind the stigmata. They separate off, and move into the fat-body, where they enlarge and assume their very distinctive features. The imaginal oenocytes are already sharply distinguishable from the larval by their small size (fig. 115, Pl. 26).

C. The Tentorium.—In the larva this consists of a transverse bar of chitin running through the head-capsole just behind the circum-oesophageal nerve strands (Text-fig. 18), from the base of one maxilla to the other; and of two much thinner bars that pass backwards from the base of the antenna to join the transverse bar some distance from its ends.

It arises late in the third day by the fusion of two pairs of tubular ingrowths of the ectoderm arising (i) at the base of the antenna just anterior to the mandible, (ii) at the hinder angle of the maxilla, between it and the labium. There is no contribution from the hinder angle of the mandible. In this respect its development resembles that of Hydrophilus (Heider), Formica and Chrysomela (Strindberg).

Because the tentorial ingrowths are tubular a transverse
canal is formed across the floor of the head-capsule (fig. 116, Pl. 26); with the chitinization of its inner face the lumen later becomes obliterated.

To the backgrowth from the antenna is attached the enlarging coelomic sac of the antennary segment (section 15), the attachment occurring just anteriorly to the developing sub-oesophageal body. At a later period we find the coelomic sac attached to the transverse bar of the tentorium; it seems, therefore, that the transverse bar is largely derived from the antennary and not maxillary ingrowth.

D. The Tracheal System.—This arises, as usual, from segmental ectodermal ingrowths in the thorax and abdomen. In Calandra these appear before shortening of the embryo has begun; they occur in the three thoracic and first seven abdominal segments (Text-figs. 12, 13, 14).

The mouths of the stigmatic ingrowths are at first very wide, but later become reduced to narrow clefts (cf. fig. 77, Pl. 24; fig. 119, Pl. 26, both representing the prothoracic spiracle).

The stigmatic ingrowths appear in close association with the coelomic sacs, against which they tend to flatten. Lehmann (1926) has already drawn attention to the close relationship between these two structures in Carausius, and discussed its possible implications.

During the period of shortening of the germ-band an approximation of successive stigmata is brought about. By the time the shortening is completed small outgrowths from the flattened ends of successive invaginations fuse and so form the rudiments of the longitudinal trunks, within which a gradually enlarging lumen arises.

The tracheal branches arise late on the third day as hollow outgrowths from the blind inner ends of the stigmatic trunks (fig. 119, Pl. 26). Prominent among these are the vessels to the head, and a large branch that passes backwards to the mid-gut.

During the fourth day all but the first and last stigmata close. The mesothoracic stigma has, throughout, been much reduced (Text-fig. 14).

By the time of emergence all the main larval vessels have developed. Throughout the larval period much elaboration of
the vessels occurs. Their anatomy in the fully developed larva is shown in Text-fig. 9 of our previous paper (Murray and Tiegs, 1935); a drawing from the first instar is given in a paper by Hozawa (1929).

18. The Nervous System.

A. Ventral Nerve-cord.—The first indication of the ventral nerve-cord appears at the time the somites are forming. The ectoderm at this period presents a pair of median thickenings (fig. 42, Pl. 22). Beginning at the anterior end and spreading backwards a differentiation now occurs within the thickenings to form an internal layer of pale neuroblasts and an external layer of smaller more deeply staining epidermal cells (dermatoblasts of Wheeler). The segregation is brought about by certain of the cells enlarging, losing their columnar shape and becoming withdrawn from the exterior to form an inner layer (the neuroblasts) while the dermatoblasts between them withdraw to the outside (fig. 43, Pl. 22).

Two continuous lateral cords of neuroblasts thus arise along the length of the embryo, the only indication of segmentation being a succession of lateral intersegmental indentations at their margins. Originally the lateral cords are not more than two to three neuroblasts in width; in later embryos as many as five rows occur in the middle of the segment, reduced to three at the intersegments. The additional cells seem to arise by differentiation out of the ectoderm, and not from already formed neuroblasts, for no proliferation of the latter has been seen.

The origin of nerve-cells from the neuroblasts\(^1\) presents but little variation from that described for other species. In Korotneff's account for Gryllotalpa (1885) and Wheeler's for Xiphidium (1893) the nerve-cells are described as forming by unequal division of the large neuroblasts, the nerve-cells thus arising being small and deeply staining, and forming columns of cells above the parent neuroblasts. In Forficula

\(^1\) Following His, the term 'neuroblast' is now generally reserved for cells that become directly converted into nerve-cells; it was originally employed for their parent cells and as such has survived in the literature on insect embryology.
several columns occur for each neuroblast (Heymons, 1895 a), in Xiphidium one. In neither species, nor in Eutermes (Strindberg) or Pieris (Eastham, 1930) does subsequent multiplication of the resulting nerve-cells take place; but in Doryphora, according to Wheeler (1893), and in Apis (Nelson, 1915) it occurs.

Early stages of nerve-cell formation in Calandra are seen in fig. 44, Pl. 22; fig. 46, Pl. 23; and fig. 67, Pl. 24; a more advanced stage appears in fig. 47, Pl. 23, the tendency of the nerve-cells to lie in columns being here clearly seen. But in Calandra, unlike most other species investigated, division of the nerve-cells occurs, and is, indeed, quite extensive; mitoses are seen in fig. 47, Pl. 23; figs. 75, 76, Pl. 24; fig. 95, Pl. 25. As it progresses the orderly alignment of the nerve-cells becomes obliterated.

As the two lateral cords enlarge and bulge on to the surface a neural groove develops between them in the position of the old gastral groove (Text-figs. 10-13; figs. 46, 47, Pl. 23; figs. 67, 68, 75, 76, Pl. 24; fig. 89, Pl. 25).

In addition to the two lateral cords the narrow median cord must be distinguished, forming the floor of the neural groove. As in other species its cells (neurogenic cells) are narrow and columnar (fig. 75, Pl. 24) except at the intersegments where rather deeply staining neuroblasts occur flanked at the sides by elongate columnar cells (fig. 93, Pl. 25). The intersegmental position of these median-cord neuroblasts is well shown in the horizontal section (fig. 98, Pl. 25). Unlike the cells of the lateral cords those of the median cord are not covered externally by dermatoblasts, but abut on to the surface of the neural groove.

Cell-division is occasionally observed among the neurogenic cells. Late during the third day they lose their columnar form and, becoming polygonal, assume the appearance of nerve-cells. They retain their original position immediately above the neural groove, and are readily distinguished from the other nerve-cells by their paler cytoplasm and rather larger size. They are now definitely part of the nerve ganglion, forming the roof and part of the lateral walls of the ventral fissure between the unfused right and left halves of the ganglion (fig. 76, Pl. 24).
ing the ventral fissure (figs. 77, 78, Pl. 24). The ‘Punktsubstanz’ increases in mass, while the axons become of immeasurable fineness. Between successive ganglia lateral connectives begin to appear, and the transverse commissures become defined.

The construction of the ganglia at this period is seen in fig. 97, Pl. 25, representing a horizontal section just under the dorsal surface of the last thoracic ganglion, of an embryo at the stage shown in Text-fig. 13. The median-cord derivative is divided by the anterior and posterior commissures into three parts—the anterior, median, and posterior zones of Graber—the last-named arising only partly from the median-cord neuroblasts, which are recognizable by their large size. To the sides lie the great masses of lateral-cord cells. Nerve axons appear with unusual clearness, and it is possible to see that the transverse commissure develops from both the lateral and median-cord nerve-cells, the latter also contributing to the formation of the longitudinal connectives.

These observations then fully confirm the statement of Heymons on Forficula that ‘the entire dorso-median part of the ventral ganglia, inclusive of certain fibres of the transverse commissures, arise from the median cord that originally formed the floor of the neural groove’.

In rather more advanced embryos the nerve-cord becomes separated from the epidermis; lateral nerves are now seen communicating with the myoblasts (fig. 78, Pl. 25; figs. 115, 117, Pl. 26).

During the fourth day the cord enlarges further. This is only partly due to growth of the ‘Punktsubstanz’, for there is still much evidence of division both of the neuroblasts and the undifferentiated nerve-cells.

The fate of the neuroblasts is hard to determine. In Xiphidi u m (Wheeler), Forficula (Heymons), and Eutermes (Strindberg) they are said to degenerate. In Calandra they diminish much in size in later divisions, and are therefore hard to distinguish from nerve-cells. Degenerated remains, if they occurred, could scarcely be distinguished from the paracytoids (section 21) which are very common in the advanced nerve-cord (figs. 116, 118, Pl. 26). In some instances degenerated cells have
been encountered at the site of the former neuroblasts, their presence suggesting that the neuroblasts do indeed degenerate (fig. 116, Pl. 26). In Hymenoptera, however, the neuroblasts survive—Carrière and Bürger (1897), Nelson (1915).

The ventral nerve-cord comprises sixteen ganglia, to which must be added a few neuroblasts at the tip of the last abdominal ganglion. The sub-oesophageal ganglion that arises by fusion of the three gnathal ganglia is much enlarged. The thoracic ganglia remain separate. On the fourth day concrescence of the last three abdominal ganglia occurs (Text-fig. 17). The abdominal ganglia thus reduced to eight become further reduced to six after the larva has emerged. In the imago further concrescence occurs, the entire nerve-cord comprising five ganglia, of which the hinder part of the third, together with the fourth and fifth, are to be reckoned as abdominal (Murray and Tiegs, 1985).

The occurrence of a few nerve-cells in the last segment (eleventh) is noteworthy; in *Leptisma* and in some Orthoptera according to Heymons a complete ganglion develops here, as also in *Chalicodoma* (Carrière and Bürger).

**B. The Brain.**—Between the neuroblasts of the brain and of the ventral nerve-cord there is no observable difference, either in regard to the time or the manner of their development; the formation of the nerve-cells from the neuroblasts and their subsequent division is also similar (cf. figs. 83, 87, 89, Pl. 25).

The three component ganglia of the brain become defined at the same time as the ventral ganglia. Their position is best seen in ventro-lateral views of entire cleared embryos. The accompanying drawing (Text-fig. 19) is from an embryo which has begun to shorten; the tritocerebral ganglion, continuous behind with the mandibular, lies postero-laterally to the stomodeum, just in front of the angle of the mandible, and, though sharply defined, is small; the deutocerebral occupies a rather larger area at the base of the antenna; the protocerebral is very large and occupies a great part of the inner surface of the head-lobe.

Histologically the tritocerebral ganglion differs from those behind it only by its median cord; the latter widens out just behind the stomodeum, is devoid of neuroblasts, and does not take any part in the formation of the mature ganglion.
The early development of the protocerebrum is best examined in longitudinal horizontal section. Fig. 92, Pl. 25, is from an embryo at about the stage of Text-fig. 19. The three component lobes of the protocerebrum are recognizable; posteriorly is the first lobe (optic ganglion), still part of the epidermis, and distinguished by its rather large cells, though neuroblasts are absent; while in front of this, indistinctly demarcated from one another by an ingrowing ridge of dermatoblasts, lie two masses of neuroblasts with their progeny of nerve-cells, separation of these two lobes being less evident than in Orthoptera and related forms (Viallanes, Wheeler, Heymons, Strindberg).

Separation of the head-ganglia from the epidermis does not occur till the end of the third day, the ganglia having now become very massive. The deutocerebrum has now merged into the protocerebrum; the tritocerebrum, however, though connected with the deutocerebrum and mandibular ganglion, retains its individuality (fig. 117, Pl. 26).

The 'Punktsubstanz' has now also begun to appear in the
various ganglia, thereby further enlarging and consolidating the brain. In the protocerebrum two such masses arise, namely in the second and third lobe, but soon merge into one; although they form on the internal surface of the ganglia, they later become completely enclosed by nerve-cells (cf. figs. 117, 118, Pl. 26). The interganglionic connectives now also develop, the circum-oesophageal strands becoming prominent (fig. 118, Pl. 26). The transverse commissures require special comment, as their development has been the subject of much discussion.

The sub-oesophageal commissure arises from the tritocerebrum. In Forficula, according to Heymons (1895 a), it develops from median-cord cells associated with that ganglion. This is confirmed by Strindberg (1913) for Eutermes, and by Carrière and Bürger (1897) for Chalicodoma; Nelson (1915) remained uncertain about the point in Apis, while in Pieris, according to Eastham (1980), it is derived not from the median-cord, but from the 'median inner cells of the tritocerebral ganglia'. Paterson (1935) in Corynodes derives it from the ganglion itself. In Calandra the median cord does not participate. This is clearly shown in fig. 117, Pl. 26, representing a frontal section of the head (transversely cut embryo), the section passing along the median cord. The latter is seen passing above on to the stomodaeum, and is quite distinct from the tritocerebral ganglia, from which axons are developing to form the commissure. The fully formed commissure is seen in fig. 118, Pl. 26.

There is a similar difference of opinion for the supra-oesophageal commissure. In Forficula Heymons derives it from the median epidermis, and this is confirmed by Eastham for Pieris and by Paterson for Corynodes. Viallanes (1891, Mantis), Wheeler (1893, Aiphiidium), and Strindberg (1913, Eutermes) derive it from the ganglion cells of the protocerebrum and deutocerebrum. In Calandra the epidermis plays no part in its formation, the commissure arising from the protocerebrum, and probably also from the deutocerebrum. An early stage in its formation is seen in fig. 117, Pl. 26; the mature commissure is shown in fig. 118, Pl. 26. In Calandra a median depression of the epidermis, similar to that figured by
The first lobe of the protocerebrum (optic ganglion) is, as already said, devoid of neuroblasts. On the third day the ganglion ‘Anlage’ begins to invaginate (fig. 92, Pl. 25), the invagination being readily visible in entire embryos as a slightly crescentic surface cleft (Text-fig. 19). Such a cleft has been figured by Patten (1888) for Acilius and by Wheeler (1893) for Xiphidium, and is described also by Viallanes and Heymons.

The fate of the invaginated cells is not known with certainty. It should be observed that in the species hitherto described it is not actually the optic ganglion that is invaginated, but rather a ridge of ectodermal cells (‘intraganglionic thickening’ of Wheeler). In Calandra, however, it is the ganglion ‘Anlage’ itself that invaginates, and this makes the cleft easier to follow in later embryos. After separation from the epidermis the ganglion, with cavity still visible despite cell proliferation, is found forming the postero-ventro-lateral part of the protocerebrum (fig. 116, Pl. 26). Heymons suspected that the invaginated cells degenerated. For Calandra this is not the case; the cavity of the invaginated mass becomes reduced to an almost imperceptible cleft, its outer (thick) wall being the optic lobe of the brain, while its inner wall forms the immediately adjacent part of the protocerebrum.

During the fourth day the enlargement of the great head muscles pushes the brain backwards some distance into the thorax. This is attended by elongation of the circumoesophageal connectives, which curve backwards over the transverse bar of the tentorium (Text-fig. 18).

C. The Sympathetic (Stomatogastric) System.—This comprises only three ganglia, pharyngeal ganglia being absent. The frontal ganglion (Text-fig. 18) is relatively large and from it nerves pass to the labrum, oesophageal dilator muscles, and to the oesophagus. The ‘recurrent nerve’ is short,
the hypocerebral ganglion being an elongate inconspicuous swelling on it (fig. 101, Pl. 25). The ventricular ganglion (Text-fig. 18) is also comparatively small; nerves pass from it on to the adjacent wall of the gut.

There are two connexions with the brain: (i) by a pair of short incurving branches from the cerebral (i.e. fused antennal, labro-frontal and ocellar) nerves, which join the frontal ganglion; (ii) short connectives between the hypocerebral ganglion and the deutocerebrum (in our previous paper the connexion was stated erroneously to be with the tritocerebrum).

In its development the stomatogastric system does not present any novel features. During the third day, in embryos in which shortening has begun, the dorsal wall of the stomodaeum loses its regular epithelial character, its cells enlarging, becoming pale, and assuming an appearance very like neuroblasts (fig. 89, Pl. 25). Unlike those of the brain and nerve-cord, however, they are not teloblasts, but divide by equal division.

The ganglia arise as the usual three median invaginations (fig. 100, Pl. 25), which after separating from the stomodaeum fuse into a continuous cord. Although the invaginations are of about equal size the first (frontal) soon outstrips the others, while the second (hypocerebral) remains small (fig. 101, Pl. 25).

The stomatogastric system is seen in transverse section in figs. 116, 117, 118, Pl. 26.

D. 'Neurilemma'.—This membrane invests the cord, brain, and sympathetic system. Although its flattened nuclei are readily seen, the membrane itself easily escapes detection, hence the difficulty of observing its development.

Korotneff (1885) in Gryllotalpa derived it from amoeboid mesoderm cells, but for most authors it is ectodermal. Wheeler (1893) believed that in Xiphidium its origin could be traced from the intraganglionic portion of the median cord, although Hatschek (1877) had already shown that the latter became an integral part of the nerve-ganglia (Bombyx). According to Heymons (1895 a) it seems to arise from the ganglia themselves by flattening out of superficial cells which during the segregation of the neuroblasts from the dermatogenic layer separate
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from the latter' (Forficula). Strindberg (1918) concluded that in Eutermes they arose from superficial cells of the ganglia, i.e. from the progeny of the neuroblasts, and this is supported by Eastham (1930 a) for Pieris.

In Calandra the neurilemma of the brain and sympathetic seems to be derived from the superficial cells of these organs. But in the ventral nerve-cord it has quite a different origin, being formed from the intersegmental portion of the median cord; in a recent paper Paterson (1935) advocates the same for Corynodes.

The cells concerned are certain long columnar cells already described (section 18 A) as flanking the intersegmental neuroblasts (fig. 93, Pl. 25). These cells become associated, late on the third day with the dermatoblasts lining the neural groove (fig. 94, Pl. 25). When these dermatoblasts now become withdrawn to the level of the sternal integument they remain associated by long filaments with the median-cord cells, the lateral spreading of the dermatoblasts causing thereby the formation of delicate partitions between successive ganglia. Fig. 99, Pl. 25, shows the partition at the thoracico-abdominal intersegment; it is from a transversely cut embryo, a fragment of the right and left hinder wall of the last thoracic ganglion being included in the section. Though best developed in the thorax, the partitions occur in all the segments. Comparable structures have been observed by Wheeler (1898) in Doryphora and Xiphidium and by Heymons (1895 a) in Forficula. In these species they form, in the thorax, apophyses for attachment of leg muscles, while in the abdomen they are said soon to disappear. But in Calandra they form the neurilemma.

In sagittal section they appear as in fig. 95, Pl. 25 (abdominal 3, 4, and 5); the preparation is from paraffin-embedded material, the shrinkage of the ganglia from the epidermis serving to accentuate the connexion between the latter and the partitions. Fig. 96, Pl. 25, is from a later embryo in which shortening is completed (abdominal 3 and 4). It is evident that the intersegmental partition, now closely investing the ganglion, has become the neurilemma, and is still connected below with the
sternal integument. This connexion survives throughout the embryonic period, but becomes less evident after hatching. Whether the neurilemmal cells that invest the lateral walls of the ganglia are derived by the spreading out of these cells, or whether they arise locally from the ganglia, present methods seem quite unable to decide.

The neurilemma of the brain also shows, in places, connexions with the adjacent epidermis, recalling, in this respect, Patten's account for Acilius (1888). When later the brain becomes pressed back into the thorax, these disappear.

19. THE MUSCULAR SYSTEM.

A description of the muscles referred to in the following account is given in our previous paper (Murray and Tiegs, 1985).

A. Muscles of Thorax and Abdomen.—These develop from two sources (i) the external walls of the somites, (ii) the subsomitic mesoderm. From the former arise, as described above (section 10), the lateral plate myoblasts—segmentally disposed masses of cells adjacent to the terga of the body-wall, i.e. dorsal to the spiracles. The subsomitic mesoderm is sternal in position and comprises (a) segmentally disposed clumps of cells, lying dorso-lateral to the nerve-cord, to the sides of the epineural sinus; (b) masses of cells in the lateral body-wall, ventral to the lateral plate myoblasts; (c) myoblasts of the appendages (thorax only) (figs. 76, 77, Pl. 24).

As the body-walls spread upwards over the sides of the egg, the lateral plate myoblasts grow dorsally, separating meanwhile into three masses, of which two are longitudinally disposed, and are the ‘Anlagen’ of the median-dorsal and dorso-lateral bands of muscle-fibres, while the third, external to these, becomes greatly elongated dorso-ventrally, and is the ‘Anlage’ of the transverse muscle-bands (fig. 78, Pl. 24; fig. 115, Pl. 26). The myoblasts themselves now become disposed into columns of elongate cells, the terminal cells being inserted on to the adjacent epidermis, usually at the intersegments. In the case of the transverse muscles enlargement of the epidermis between the points of insertion draws out the columns of myoblasts to their definitive length.
The ventral muscle-band develops from the most lateral portion of the subsomitic mesoderm; from the latter arise also, it seems, the first and second oblique muscle-bands, as well as the most ventral part of the transverse muscles, the tergal part of which is derived from the lateral plate myoblasts.

The strongly developed third system of oblique muscles, i.e. most internal, arises from the clumps of subsomitic mesoderm cells lying dorso-lateral to the nerve-cord. A few of the most anterior cells of these masses are in direct contact with a ridge of epidermis that projects inwards intersegmentally to the side of the nerve-cord, while other cells at the hinder ends of these masses, bending outwards, become associated with the epidermis of the lateral body-wall one segment behind. With the spreading of the lateral body-wall over the sides of the egg, late on the third day, the epidermis between these two regions of attachment becomes much enlarged, with the result that the clumps of myoblasts become drawn out into long obliquely running columns of cells (fig. 115, Pl. 26). Thus arises the innermost system of oblique muscles.

In the prothorax occur certain muscles specially adapted for movement of the head. The two head depressors develop from the lateral plate myoblasts of the prothorax (fig. 119, Pl. 26), while the levator capitis is formed from the clumps of myoblasts nearest the nerve-cord.

The myoblasts that occupy the cavities of the thoracic appendages remain dormant till metamorphosis. In the larva they form clumps of cells associated with the imaginal disks of the legs (fig. 102, Pl. 25); from them arises, in the imago, the intrinsic musculature of the legs.

B. Muscles of Head.—The larval antennae are devoid of muscles, a clump of associated myoblasts, derived from the antennary mesoderm, remaining dormant till metamorphosis, when they form the flexor and extensor muscles of the antennae.

From each of the gnathal segments a pair of muscles—flexor and extensor—develop in association with each appendage. (In our previous paper we did not describe muscles in association with the labium; they have been detected in the present material and comprise very minute muscle-fibres, 2 to 3 in number).
In the mandibular and maxillary segments, as already recorded, coelomic sacs are absent and the mesoderm is unsegmented. It occupies the cavities of the appendages and spreads a little outwards on the body-wall. With the beginning of shortening of the germ-band this mesoderm becomes much enlarged, partly by addition of cells from the cavities of the appendages.

The tendon of the great flexor muscle of the mandible arises shortly after this. It develops as a large hollow ingrowth of the ectoderm at the hinder corner of the mandible. This ingrowth comes into association with the rapidly enlarging adjacent mass of mesoderm cells (myoblasts) which now arrange themselves into radiating columns of cells from which the muscle-fibres are formed (Text-fig. 18; fig. 105, Pl. 25; fig. 116, Pl. 26). Chitinization of the tendon occurs late on the fourth day. The much smaller extensor muscle of the mandible arises in the same way, anteriorly to the flexor.

The muscles of the maxilla take their origin from the crossbar of the tentorium. The association is established by the maxillary component of the tentorium (section 17 C) drawing the myoblasts with it along its path of invagination. The minute labial muscles come from the subsomitic mesoderm.

20. THE GONADS.

The origin of the sex-cells at the hinder pole of the blastoderm has been described in section 4.

With the formation of the germ-band, the sex-cells cease to appear at the surface, becoming overgrown by the germ-band as it bends upwards over the hinder pole of the egg. The elongation of the germ-band carries them by about the middle of the second day to the anterior pole of the egg, where they form a conspicuous clump of rather pale cells just below the surface. Stages in this migration are shown in Text-fig. 6.

From this position at the hinder end of the germ-band the mass moves forwards again, in the developing abdomen, as the proctodeum elongates. Early stages of this are shown in figs. 60 and 61, Pl. 23; by the end of the second day the mass has moved forwards almost to the eighth segment (fig. 64, Pl. 23).
At about this period the germ-cells are coming into close relationship with the adjacent coelomic sacs, the masses thereby dividing into right and left halves. An early stage of this is shown in the serial sections given in fig. 67, Pl. 24; it will be observed that the division proceeds from before backwards.

The inferior portion of the splanchnic walls of the more posterior abdominal coelomic sacs consists of small, rather scattered cells, without any regular epithelial alignment. From these arises the investing sheath of the gonad. As the germ-cells come into relation with the coelomic sacs, these cells become indented by them into the coelomic cavities, which thus become obliterated (fig. 67 n, fig. 68, Pl. 24). The investing sheath which the germ-cells thus acquire consists at first of scattered cells only, though in later embryos these become consolidated into a continuous membrane (cf. fig. 75, Pl. 24; and fig. 104, Pl. 25, both taken at the level of the seventh abdominal segment; also fig. 103, Pl. 25).

This penetration of the sex-cells into the coelomic sacs occurs in the middle of the third day, just before the beginning of shortening of the embryo, at a time when the cavities of the sacs have already become confluent, and when differentiation of the walls is beginning. From the ninth, eighth, and seventh sacs, where the penetration occurs, they migrate forwards as a compact cord of cells, about three or four segments in length, till they reach, at the end of the third day, the third abdominal segment.

By the time the gonads reach that level the fat-body has become well defined. The gonads now become spherical masses, and lie amongst the fat-cells dorsally in the haemocoele, whither they have been carried by the upward spread of the body-walls over the sides of the egg. There is no connexion with the developing heart as in Orthopteran embryos.

During the fourth day the gonads move back to about the seventh abdominal segment (Text-fig. 17).

The sexual ducts arise late on the fourth day from the mesodermal cells that ensheath the gonad. These cells have, by now, considerably increased in number, and are conspicuous on the inferior surface of the gonad. Shortly before the larva emerges
these cells grow downwards as a pair of solid stalks to the base of the ninth segment, where they impinge on the epidermis. Shortly after emergence of the embryo the gonad becomes completely encased by fat-body.

Except for the presence of mycetocytes in the ovary, there is no perceptible difference between the latter and the testis.


Reference must now be made, in this concluding section, to a peculiar cytological phenomenon—paracytoid formation—which is displayed by cells of the embryonic tissues at various phases of the insect's development.

In our previous paper on the metamorphosis of *Calandra* this phenomenon was described under the name of 'chromatic globule extrusion', and its similarity shown to the process observed by Poyarkoff twenty-five years earlier in the metamorphosis of *Galerucella*. It consists in the extrusion of minute pieces of chromatin from the nucleus into the cytoplasm, where they apparently swell, cohere, and after receiving an investing film of cytoplasm, become cast out from the cells into the blood. It occurs not only for cells which, like the epidermal and tracheal cells, have contributed to the formation of the larva, but also for cells which have remained embryonic during larval life, e.g. the imaginal myoblasts. We now find the same process occurring during the development of the embryo within the egg; examples are clearly seen in the following illustrations: fig. 34, Pl. 22; figs. 51–60, Pl. 23.

The formation of these globules in the embryo is best examined in the ectodermal cells, at a period prior to formation of coelomic sacs. A cell with well-developed globule is shown in fig. 80, Pl. 23. The globule is large and rounded and is contained within a vacuole. Sometimes it stains uniformly deep with haematoxylin; more often it exhibits one or more deeply chromatic clumps lodged within a pale spherical matrix. In depth of staining the chromatic clumps compare with the chromatin of dividing nuclei, and much exceed that of the resting nuclei. They give the specific chromatin reaction with Feulgen's reagents, while the surrounding pale matrix appears green if 'light green'
counterstain is applied. Whether it is cytoplasm, or extruded plastin material, needs investigation.

Although, then, largely of nuclear origin, the chromatic globules do not arise in their mature form from the nucleus, for the nuclei present, at most, only small chromatic inclusions (nucleoli ?) and even these are usually not seen. If it is legitimate to reconstruct their manner of formation from their appearance in other cells, then it would seem that comparatively small particles are extruded into the cytoplasm from the nucleus, a film of cytoplasm (?) condensing round them, while at the same time the chromatic particles swell and eventually cohere into larger drops. The occurrence of swelling must be inferred from the fact that the globules may, at times, exceed a normal nucleus in size.

Eventually the globules are extruded from the cells into the intercellular spaces, whence they find their way mostly into the yolk. Here they are to be seen lying in small clusters, particularly at the posterior and anterior ends of the germ-band (figs. 51, 52, 54, 60, Pl. 28). Some of them have much enlarged, either by further swelling or by fusing with other globules.

In the extensive literature on insect embryology reference is frequently made to a peculiar phenomenon of 'Paracyten' formation, consisting in the extrusion of modified cells (Paracyten) from the embryonic tissues into the yolk. They were first described by Heymons (1895 a) in Orthoptera and Dermaptera, and (1901) in Scolopendra, and have since been reported by other authors—Friedrichs (1906), Schwartze (1899), Strindberg (1913), Wiesmann (1926); while the frequent references to passage of degenerate cells into the yolk probably relates to the same. In appearance the Paracyten are like the 'chromatic globules' above described; they are, however, in all cases, transformed cells, and not of intracellular origin. Such Paracyten are occasionally seen also in Calandra (fig. 82, Pl. 24), but are of infrequent occurrence.

Friedrichs has, however, described in the development of Donacia a process which seems to be identical with that observed in Calandra. From the true Paracyten he distinguishes these bodies of intracellular origin as 'Paracytoids'.
They arise from the germ-cells, ectoderm, and mesoderm, and are cast out into the yolk. Except that they are stated to arise also from yolk-nuclei in Donacia they resemble the chromatic globules of Calandra so closely as to justify the adoption of Friedrichs' designation for the latter.

As to their significance nothing is known. They are not an expression of cell-degeneration, for the cells containing them are otherwise normal in appearance, and, indeed, are not infrequently seen in mitosis (fig. 81, Pl. 24). Nor do they seem to be concerned with digestion of yolk, as suggested by Friedrichs, for similar globules are shed by the serosa and amnion into the extra-embryonic fluids (fig. 64, Pl. 23). Nor do the time and place of their occurrence yield any clue; they appear about the middle of the second day in the ectoderm, and especially, but by no means exclusively, at the anterior tip of the germ-band; also in the sex-cells and embryonic membranes. In some parts, such as the head lobes, they may, indeed, be surprisingly numerous. Later they appear, though usually only sparsely, in the mesoderm and the mid-gut 'Anlage' (cf. figs. 51–5, Pl. 23). During the third day they are seen within various organs which are arising at the time (fat-body, corpora allata, tracheae, splanchnic muscle, and particularly the nervous system). Their degree of prevalence in any tissue is not, however, a real measure of the frequency of their formation, for their apparent preponderance in the nerve-cord and brain of later embryos is probably due to the difficulty of eliminating them out of such massive organs into the blood. Within the brain they occur even after the larva has hatched.

There is no evidence for their association with any visible histological differentiation of tissues; while Poyarkoff's suggestion—that they are the expression of a dedifferentiation of specialized cells preceding redifferentiation into those of the imago—is excluded on the ground of their occurrence in the developing egg.

Are the paracytoids perhaps a device for maintaining a nucleo-cytoplasmic ratio in rapidly multiplying cells?
SUMMARY.

1. In the maturation of the egg, although post-reduction appears to occur, there is actually pre-reduction, but much obscured owing to a separation of precociously split chromosomes late in the first meiotic anaphase. A temporary separation of conjugated chromosomes also precedes the first meiotic division.

2. Cleavage (non-synchronised) follows rapidly upon fusion of male and female pro-nuclei. The cleavage-cells spread through the yolk, apparently by their own activity. A cleavage pattern, though not directly observable, is to be inferred on theoretical grounds.

3. The cleavage-cells become drawn into the periplasm by a centrifugal flow of the cytoplasm. Upon entering the periplasm, or just before this, the nuclei divide. Early clearages in the blastoderm are therefore synchronised. Later the synchronisation disappears, though other remarkable forms of co-ordination have been encountered. As the blastoderm matures, the cells, now much diminished in size, develop first lateral and then inner cell-walls, the latter within the secondary periplasm.

4. The yolk-cells are derived from a small number of cells that do not enter the periplasm. They divide apparently solely by mitosis. The blastoderm does not contribute appreciably to their number.

5. The germ-cells are part of the blastoderm, and protrude prominently at the hinder end. Later they become withdrawn level with the blastoderm. They early become infected with bacteria from a large bacterial mass in the adjacent yolk. The mycetocytes of the ovary arise at this time by migration of adjacent blastoderm-cells into the bacterial mass.

6. The germ-band arises, as usual, by a dorsal thinning and a ventral and lateral thickening of the blastoderm. Beginning at the anterior end the median and two lateral plates become demarcated, the latter then invaginating with formation of a temporary gastral groove. The invaginated cells form the inner layer, which is entirely mesodermal.
7. The germ-band, meanwhile, grows over the hinder pole of the egg and extends to the anterior end, carrying the germ-cells with it. Associated with this is a peculiar method of amnion-formation due to deep invagination of the germ-band into the yolk; on the ventral surface of the egg the amnion arises by downgrowth of folds along the margin of the germ-band.

8. Segmentation of the germ-band proceeds from before backwards, without the formation of macrosegments. A vanished twelfth segment is inferred for the abdomen. The appendages develop approximately in order from before backwards. The labrum appears later. In the abdomen there are no appendages. Shortening of the germ-band occurs on the third day, and thereafter the larval form is gradually assumed, the lateral body-wall growing upwards over the yolk. The formation of the head is described in detail. The thoracic appendages merge into the body-wall in the advanced embryo, and form, then, the imaginal disks of the legs.

9. Amnion and serosa do not rupture, but form a permanent enclosure for the embryo.

10. The stomodaenum marks the anterior limit of the invaginated inner layer, the pre-oral mesoderm arising by the spreading forward of cells from behind the stomodaenum. The post-oral mesoderm differentiates into a median unsegmented sheet of cells and two lateral rows of somites, extending from the labial to the tenth abdominal segment; elsewhere the mesoderm remains unsegmented. With the exception of the tenth abdominal, these somites expand into coelomic sacs. A coelomic sac forms later in the antennary segment also.

11. On the third day, the cavities of the coelomic sacs having become confluent, differentiation proceeds: the splanchnic wall forms the splanchnic muscle of the gut; the inferior wall becomes the fat-body; the dorso-lateral wall forms the heart tissue, while the lateral wall becomes resolved into the lateral plate myoblasts, from which much of the somatic musculature develops.

12. The remaining somatic muscles are derived from the sub-somitic mesoderm, into which is incorporated most of the median unsegmented mesoderm.
13. With the withdrawal of the median mesoderm the epi-
neural sinus is formed between the yolk and the nerve-cord; by its enlargement the haemocoele is developed.
14. The stomodaeum and proctodaeum arise as simple in-
growths of the outer layer, the latter occurring at the posterior limit of the germ-band.
15. The mid-gut has a bipolar origin. It forms from the blind ends of the stomodaeum and proctodaeum, quite inde-
pendently of the inner layer. The mid-gut ‘Anlagen’ grow towards one another and meet in the first or second abdominal segment. They eventually wholly enclose the yolk.
16. The difficulty of reconciling such facts with the germ-
layer theory is discussed.
17. The malpighian tubes arise from the proctodaeum, the first pair much preceding the other two in time of development.
18. The remarkable adaptation of the development of the intestine to the bacterial symbiont is described.
19. The sub-oesophageal bodies arise from the mesoderm just anterior to the mandible, then become part of the mid-gut wall, but later lose association with it. They survive even into the imago.
20. The corpora allata do not arise from the ectoderm, but from the inferior wall of the antennary coelomic sac.
21. The dorsal blood-vessel and associated tissues arise from the dorso-lateral walls of the two rows of coelomic sacs, which meet in the mid-line above the gut and enclose a tube. The cephalic aorta is derived from the antennary coelomic sacs. The blood-cells are formed exclusively from a narrow median ridge of mesodermal cells above the nerve-cord.
22. The tracheal system arises from ten pairs of stigmatic invaginations from the prothoracic to the seventh abdominal segment. At their blind ends these expand to form the two main longitudinal vessels; from their blind ends the branching tracheae to the tissues also grow out. In the advanced embryo all but the first and last stigmatic openings close.
23. The nerve-cord arises early as a pair of ventral thickenings of the outer layer (lateral cords), within which the cells differentiate into dermatoblasts and neuroblasts. The latter are
teloblasts, and bud off a succession of nerve-cells, which themselves further divide. The median cord, between the two lateral cords, contributes to the formation of the ganglia; its intersegmental neuroblasts attach themselves to the posteromedian wall of the ganglia, while the intra-segmental (neurogenic) cells form the roof of the completed ganglia, their axons contributing to the formation of the transverse commissures and longitudinal connectives. Sixteen ganglia form in the nerve-cord, the first three uniting into the sub-oesophageal ganglion, while the last three abdominal also fuse.

24. The three component ganglia of the brain are clearly defined in the embryo. In the protocerebral ganglion the usual three lobes are seen. The optic ganglion does not contain neuroblasts and arises by invagination from the surface. Unlike the ventral nerve-cord, there are no median cord components in the brain.

25. The stomatogastric system arises as three invaginations from the roof of the stomodaeum.

26. The neurilemma of the ventral nerve-cord is derived from certain intersegmental median-cord cells. In the brain and sympathetic it is derived from the ganglia themselves.

27. On the third day the germ-cells move forward in the abdomen, enter the hinder coelomic sacs and press forward as a solid cord of cells to the third abdominal segment. They form here a spherical mass of cells and are now encased in fat-body. The genital ducts arise from splanchnic mesoderm cells ensheathing the gonads.

28. A peculiar cytological phenomenon—paracytoid formation—appears at the time of germ-band formation and in later phases of development. It is indistinguishable from the 'chromatic globule extrusion' already described from the metamorphosis of Calandra. Its significance is unknown.
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