XV - THE DIGESTIVE SYSTEM

The alimentary canal of insects consists of three major portions, viz., fore-gut, mid-gut and hind-gut. Embryologists are in general agreement regarding the ectodermal origin of the fore- and hind-guts but considerable difference of opinion exists on the origin of the mid-gut epithelium.

1. The Stomodeum

In *A. proxima* at the age of about 22-hours a shallow ectodermal depression appears a little behind the anterior end of the embryo. This is the earliest indication of the stomodeal invagination. As it grows it acquires a tabular form with its blind end directed obliquely (Plate 3, Fig. 21 and Plate 4, Fig. 28). Its external opening represents the future mouth. As development progresses and the head becomes well defined the mouth moves from the entero-dorsal to its definite ventral position. This change in form is accompanied by a considerable elongation of the stomodeum. At about 22-hours the blind end of the stomodeum expands a little and its wall becomes infolded so as to form four internal ridges (Plate 8, Fig. 55), which present in transverse section a star-shaped appearance. This portion ultimately contributes to the formation of the stomodeal valve. The cells forming the floor of the expanded blind end thin out to form a delicate limiting membrane which serves as a temporary partition between the fore-gut and the developing mid-gut (Figs. 40 and 55).

The development of stomodeum in *A. proxima* is similar
to that of *Apis* (Nelson, 1915) except in one important respect. In the honey bee, Nelson reports that the limiting membrane is formed from cells of the anterior mid-gut rudiment, which at an early stage become closely associated with the blind end to form a delicate membrane. In *A. proxima* it is formed from the stomodeum itself and is, therefore, ectodermal in origin.
2. The Mid-gut

(i) The anterior mid-gut rudiment (Plate 3, Fig. 21 and Plate 4, Fig. 28, mag.).

At the age of about 16-hours the anterior mid-gut rudiment develops a little behind the anterior tip of the germ band. At the place where it begins to form the cells of the germ band become irregularly distributed. This is followed by active proliferation which continues for some time and the liberated cells become heaped up to form a rounded swelling. As the rudiment increases in bulk by further addition of cells it becomes compact. The cells of the rudiment are generally rounded and stain uniformly with the rest of the germ band. While these cells are being proliferated the inner layer begins to be formed from the median portion of the germ band. These mesodermal cells soon spread over and anteriorly become confluent with the anterior mid-gut rudiment. It is at this stage that the cells of the rudiment and those of the inner layer are difficult to identify because of their similarity in form, size and their staining reactions. A few hours later, when the ribbons of cells grow out from the rudiment the distinction between the two types of cells becomes apparent (Plate 3, Fig. 22, mag). During this period of development the germ band rapidly increases in length and carries along with it the anterior mid-gut rudiment to a more dorsal position on the anterior pole of the egg. The stomodaeum develops at the site of the rudiment and as it grows to form a blind tube, the latter is carried further into the yolk.
(ii) The posterior mid-gut rudiment (Plate 2, Fig. 16 and Plate 7, Fig. 49 pmg.).

Early stages of formation of the posterior mid-gut rudiment are difficult to follow because of the manner in which the caudal extremity curves round the posterior pole of the egg and comes to lie on the dorsal surface. It is, however, fairly certain that the posterior rudiment also arises in the same manner as its counterpart in the anterior region. It is distinctly larger than the anterior rudiment and lies in the last abdominal segment. With the elongation of the germ band it is carried to the dorsal side and occupies the fold formed by the invaginated posterior extremity. The cells of the mesoderm and those of the posterior rudiment become confluent and run indistinguishably into one another.

It has already been pointed out that the stomodaeeum and proctodaeum as they grow, push these rudiments to a more internal position. Finally, the anterior rudiment comes to lie as two masses along the ventro-lateral sides of the stomodaeeum and the posterior rudiment appears as two groups of cells associated with the blind end of the proctodaeum (Plate 3, Fig. 23, pmg). The cells of the two rudiments are identical. As development progresses the posterior tip of the anterior mid-gut rudiment forms two bands (ribbons) of cells which grow posteriorly between the yolk and the splanchnic layer. A similar pair of ribbons is formed from the posterior rudiment. These paired ribbons grow in opposite directions and finally unite. The cells soon begin to spread out ventrally and enclose the yolk completely from the ventral side (Plate 5, Fig. 32). Dorsally it remains uncovered by the mid-gut cells until such time as the definitive dorsal closure is completely.
formed. At about 52-hours the lateral edges of the embryo begin to grow upwards along the sides of the yolk to effect the dorsal closure. Along with this, the fast spreading mid-gut cells from opposite sides rise upwards and with the dorsal closure of the body (56-58-hours) they unite mid-dorsally to enclose the yolk within a cellular envelope from which the mid-gut epithelium is derived (Plate 5, Fig. 36).

The cells of the mid-gut undergo several changes before they attain their definitive form. At the age of 70-75-hours tall vacuolated cells appear and pseudopodia-like outgrowths are formed from their free ends (Plate 6, Fig. 42). Before the larva hatches a considerable amount of yolk has been used up and only a few scattered spherules can be observed in the gut. Some of the yolk spherules lie in close contact with the pseudopodia and are probably engulfed by them.

The question of germ layers in insects is intimately connected with the origin of the mid-gut epithelium and the role played by the yolk cells. Considerable amount of literature has accumulated on the subject and it is to be noted that in spite of numerous attempts to homologize the germ layers no uniformity of interpretation has been arrived at. In many cases as Eastham (1930) remarks:

"... want of agreement rests on differences of opinion rather than differences of concrete results obtained..."

Aside from the difficulties that may arise due to imperfect technique, the embryologists are sometimes confronted with certain unsurmountable experimental difficulties. The writer can not
refrain quoting Demerec (1950) in this connection. He says:

"Those working on insect embryology are severely restricted by their inability to alter the position of cells at will, with operative techniques, in order to ascertain the total as well as the actual potencies of the cells in various regions of the organism at the time separation into parts, that is, layers, occurs. Because of this lack, the insect embryologist is limited therefore to interpretations of the specificity or non-specificity of the germ layers based primarily on observation rather than on observation plus experiment."

At present it is not intended to add anything new to the existing germ layer controversy, but it seems more profitable to interpret the present observations in the light of the views expressed by earlier workers. It will be seen that several writers (Nelson, Eastham, Roonwal and others) have adequately reviewed the subject and there is a discussion on this controversial matter in the book on insect embryology by Johannsen and Butt (1941). Eastham's (1930) review is particularly enlightening. The following important views regarding the origin of the endoderm in insects have been summarily adopted from his review. The position according to him can be reduced to three different expressions of opinion.

(1) Mid-gut originates from anterior and posterior mesenteron rudiments.

This view has several supporters. Some of the writers viz., Henson (1932) Shafiq (1954) and others maintain that these rudiments arise through a combination of mesodermal and endodermal layers and should, therefore, be considered as mesoendodermal in origin. On the other hand, Carrière and Bürger (1897) in Chalicodoma claim that these rudiments arise independently of the mesoderm. This view was partly accepted by Nelson (1915) in the honey bee and is endorsed by the present writer in A. prxima.
(ii) Endoderm is embryonic and is represented by yolk cells. The larval mid-gut is derived from the blind ends of the stomodaenum and proctodaenum and should, therefore, be considered as ectodermal in origin.

Observations of several workers on Coleoptera, Lepidoptera and Orthoptera are in favour of this view.

(iii) True endodermal mid-gut exists and is represented by yolk cells.

Eastham maintains that the theory of mid-gut originating from yolk cells has been questioned several times by workers on insect embryology and should, therefore, be left out of consideration. It should, however, be pointed out that in recent years the interest in the yolk cell origin of the mid-gut has been renewed by the observations of Stuart (1935) on the grass-hopper, Melanoplus differentialis. He has shown that before hatching occurs the yolk cells (vitellophags) move towards the periphery, divide and give rise to definitive mid-gut epithelium. From the observations on the development of *A. proxima* it is pretty certain that the yolk cells do not take part in the formation of mid-gut epithelium. Although, yolk cells are present in large numbers at the time of mid-gut differentiation and occasionally few cells may be observed lying close to the developing mid-gut cells, yet neither their number nor position can warrant any favourable conclusion. They can be clearly distinguished from the mid-gut cells by their large size and the form of the nuclei.

The possibility of the mid-gut originating from the blind ends of the stomodaenum and proctodaenum in *A. proxima* is
equally improbable since the mid-gut rudiments are formed much before the latter appear. The mid-gut in this sawfly is bipolar in origin and this conclusion is in agreement with the findings of Carriere and Burger (1897) in *Chalicodoma*, Nelson (1915) in the honey bee and Shafiq (1954) in the gooseberry sawfly.
3. The Proctodaeum

The proctodaeum in \textit{A. proxima} is formed before the stomodaeum. Another interesting feature is that it does not originate in the usual way as an ectodermal invagination but is contributed both by a portion of the amnion and the germ band.

It has already been described earlier that the germ band after its establishment begins to elongate rapidly. Its posterior extremity grows faster and at the age of 18-20-hours comes to lie on the dorsal surface deeply buried into the yolk. This invaginated posterior end carries all along its way the amnion which, as already stated, extends as a continuous membrane on the ventral side of the embryo (Plate 2, Fig. 16). During the next one or two hours the amnion ruptures on the ventral side and ultimately disappears, leaving behind a small fragment which is continuous with the invaginated end of the germ band. The free end of this amniotic rudiment soon grows over dorsally to form the provisional dorsal closure (Plate 3, Figs. 17 and 23 pdc). The invaginated portion of the amnion and the germ band in due course of time gives rise to the proctodaeum. This amnio-proctodaeal invagination, as it may now be called, further deepens and in sagittal sections presents the form of a bent U with its ventral wall thicker than the dorsal one. Soon, however, both the walls become uniformly thickened. Between these two walls is the posterior amniotic cavity (Fig. 16, pamcav) which shall be subsequently termed the amnio-proctodaeal cavity, as it is from this cavity that the lumen of the proctodaeum develops. The proctodaeum progressively differentiates and lies on the dorsal side of the
egg with its external opening (the future anus) pointing dorsally. With the shortening of the embryo and the consequent shifting of the caudal extremity the proctodaeum undergoes several changes of position with respect to the body axis. Finally, at the time when the larval form is recognizable it becomes parallel to the long axis, with the anus pointing anteriorly. Towards its blind end, at the age of about 34-hours, a thin limiting membrane develops which undoubtedly arises by the thinning out of cells of the proctodaeal wall and has, therefore, been regarded as ectodermal in origin. It screens off the hind-gut from the mid-gut but late in the embryonic life it ruptures to allow free communication between the former and the latter.

The early development of proctodaeum in *A. proxima* is very similar to what has been recorded by Butt (1949) in *Oncomeltus* and more recently by Sender (1955) in *Pyrrills*. In both these insects the dorsal wall is claimed to be amniotic in origin while the ventral one is contributed by the embryo proper. In the other Hymenoptera the proctodaeum develops as a simple invagination in common with the majority of insect embryos.