POST-EMBRYONIC CHANGES IN **Melipona quadrifasciata anthidioides** Lep. **IV**. DEVELOPMENT OF THE DIGESTIVE TRACT (1)

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MODIFICAÇÕES PÓS-EMBRIONARIAS EM **Melipona quadrifasciata anthidioides** Lep. **IV**. DESENVOLVIMENTO DO TRATO DIGESTIVO

RESUMO No presente trabalho estuda-se o desenvolvimento post-embrionário do tubo digestivo de **Melipona quadrifasciata anthidioides.** Cada uma das três partes do canal alimentar (estomódeo mesentério e proctódeo) são tratadas isoladamente. O aspecto larval de cada porção é descrito antes de expor as transformações que ocorrem posteriormente. A maioria das transformações post-embrionárias do tubo digestivo ocorrem durante a pupação, mas algumas ocorrem já na larva ou durante a fase de pré-pupa. A Tabela I é uma sinópse das mudanças pos-

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embrionárias que ocorrem nessas três fases. Como pode ser visto as mudanças se iniciam 2 dias após a eclosão da larva e estão completas quando a pigmentação do corpo se inicia na pupa, ou seja, 6 dias antes da emergência.

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ABSTRACT The present paper studies the post embryonic changes on the digestive tract of the stingless bee **Melipona quadrifasciata anthidioides.** Each of this three parts of the alimentary canal (fore, mid, and hindgut) were treated isolated. The larval features of each part are described before to relate the later transformations. Most of the post-embryonic changes in the digestive canal occusr during pupation but a few take place in prepupae and even larvae. The table I is a synopsis of the post membryonic changes. As can be seen the post embryonic changes begin 2 days after the egg hatching and are finished when the eye pigmentation is completed (brow eyed pupae i. e, 6 days before emergency.

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The fundamental structure of the alimentary canal of insects has been studied by many biologists on account of its volume and facility to separe from the other tissues. In the hymenopteran the digestive tract was also object of numerous papers. Most of them refer to the embryological (Nelson, 1915; Schnatter, 1934), larval (Nelson, 1924; Snodgrass, 1925) and adult (Bordas, 1905; Snodgrass, 1925; Cruz-Landim and Rodrigues, 1967) stages. The pupal stage, i. e, the metamorphosis of the digestive canal was described by Evenius (1926), Oertel (1930), Green (1933), Lotmar (1945), and Dobrosky (1951), among others. Except the papers of Green **(Vespa vulgaris)** and Schmidt **(Formica polyctena)** others refer to the honeybee.

The most controverted points in the events taking place during post-embryonic development of the gut, are the origin of the peritrophic membrane (Dehn, 1937; Hering, 1939; Kusmenko, 1940; Day and Waterhouse, 1953), Malpighian tubules (Nelson, 1915; Trappmann, 1923; Henson, 1933; Weil, 1935; Kusmenko, 1941) and rectal pads (Evenius, 1933; Lotmar, 1945; Dobrovsky, 1951).

The present paper describes the post-embryonic changes in the digestive tract of **Melipona quadrifasciata anthidioides**, a stingless bee. We intend to compare our findings with those on literature, mainly with the development of **Apis**.

In spite of the fact that we have made a very detailed study of the larval, prepupal and pupal stages, we will refer mainly to the development of the proventriculus, ventriculus, Malpighian tubules and rectal pads. We do not expect great differences between the post-embryonic changes in the gut of **Apis** and **Melipona** but we hope that additional information could be gained on this subject by this work.

MATERIAL AND METHODS

The material used consisted in normal specimens from larvae, prepupae and pupae of Melipona's workers. By observing the brood, the ages of the larvae were exactly determined. The **Melipona** larva hatches at the end of five days from the deposition of the egg. We took larvae each three hours after hatching. From the prepupal stage we took samples from the beginning and ending of the stage (this stage takes 3 days — Cruz-Landim, 1966). The age of the pupae were determined approximatly by the eye and body pigmentation (Cruz-Landim and Mello, 1968) and this process is more advantageous than to mark the cell and wait until the age increasing because one can have the pupae any time they are needed. We used white bright-pink pink, red, brown eyed pupae and pupae in the beginning of the body pigmentation.

We studied mainly the histological changes in the gut, but we also dissected some prepupae and pupae for morphological analysis.

For dissection the specimens were anesthetized by chilling and dissected in 0,6% saline solution.

For histological purposes Bouin's fluid, was used and 10μ thick sections stained by hematoxylin and eosin.

RESULTS AND DISCUSSION

In the **Melipona's** colony the queen lays her eggs in a cell previously filled with food in such a way that the future larvae will swim in it until pupation. Just after the laying of the egg. the cell is sealed. The food however, is deposited into the cell by the nurse workers in layers of different composition from botton to top (Sakagami and Zucchi, 1966). Thus, the larvae will eat different kinds of food as increase in age, just in the same way as the honeybee.

The alimentary canal is adjusted to serve the alimentary function and its structure is modified as the food changes.

The foregut or stomodaeum (fig. 1A and 2) starts in the larval mouth that leads immediately into pharynx and ends in the cardiac valve projected into the midgut.

The pharynx is a very short tube, whose dorsal wall is depleted in a longitudinal fold (epipharynx) in which is situated the only musculature of the foregut anterior part. The figure 3 shows a longitudinal section through the epipharynx where the muscles can be seen. These muscles are longitudinal and transverse. The longitudinal are in the bottom of the groove beneath the cross musculature. Besides these muscles there are several sets of extrinsic muscles (dorsal and ventral), associated to the pharynx. The extrinsic muscles run from the exoskeleton directly to the epithelial cells. The epithelium of the epipharingeal groove (fig. 3) is tall, made up of cylindrical cells, while the pharynx wall is thin and made up of squamous cells.

The larval eosophagus (fig. 4) is a short, narrow tube, not sharply differentiated from the pharynx. The epithelium lining the tube is columnar. The muscular sheath consists of circular and longitudinal muscle fibers, both well developed.

During the larval life the cardiac valve remains opened into the ventriculus (fig. 2 and 5). The cardiac valve consists of two layers of cells apposed together. The cells are tall almost filiform with the nuclei in the apical part.

The epithelium lining the foregut is continuous with the body wall as also the epithelial cells cuticle. In the cardiac valve this cuticles loose from the cells and appear as a wrinkled material (fig. 5) that enters the ventriculus.

A narrow ring, four cells wide (fig. 6), is a true connection between the oesophagus and the midgut in the larval food tract. Those are narrow, tall cells, bigger than the valvular cells but smaller than the ventricular cells, and constitute the "imaginal ring" often referred to by many investigators.

The foregut remains unchanged during all the larval stage.

The prepupae stage in **Melipona** takes 3 days (Cruz-Landim, 1966) and during this time the head, thorax and abdomen are differentiated, as well as, their appendages. During this time no significant morphological changes take place in the foregut (fig. 1B). However, sections from the oesophagus (fig. 7) show some reorganization in the epithelium and a increase in the thickness of the muscular sheath. In the eosophageal lumen, at this time, it can be seen chromatic globules resultant from some cells degeneration.

During pupation the constriction dividing the thorax from abdomen becames deeper taking finally the imago aspect. The foregut does not suffer a real metamorphosis in the sense of degeneration of the larval organ and organization of a new one during pupation. The larval foregut differenciates and reorganizes during pupation without breaking down.

In white eyed pupae a slight dilatation appears in the foregut (fig. 1C) a little above the ventriculus. This is the first step in the development of the "honey stomach" or crop. Caudad from the crop the foregut continues as a short straight tube, without any morphological differentiation. Cephalad the tube also continue unchanged except by the appearence of the hypopharingeal gland buds in the pharynx (fig. 17). The figures 8 to 16 shows a serie of cross section through the region caudad to the crop dilatation. The passage into the ventriculus is closed during the pupal life, as seen in the figures 11 and 12. The proventriculus dfferentiation starts in this phase, althought it can not be seen morphologically. Some mitosis occurs in the tube between the crop and cardiac valve (fig. 16) and in the transition from this tube to the lumen already appears square (fig. 16).

The foregut differentiation proceeds and when the pupae eyes start to show pink shades the proventricular region of the foregut is already morphologically visible (fig. 1C), the crop is enlarged a little and hypopharingeal glands appear as slender short tubes connected to the pharynx. The sections show that the crop walls (fig. 18) remain the same as in white eyed pupae but the proventricular region bears thick walls in which numerous mitosis can be seen (fig. 18 and 19). The proventricular walls are thicker in the anterior portion and thinner close to the ventriculus (fig. 18). The anterior region will originate the proventriculus bulb and cross sections from this part already show the four lips formed (fig. 20). The posterior part will originate the proventriculus neck. In this phase most of the mitosis occur in the further proventriculus neck in order to provide its elongation. The circular and longitudinal layer of musculature remain well delimitated during all these transformations. The proventricular bulb is made up mostly of muscular tissue, but those muscles arise from division of myoblasts inner to the organ muscular layer.

The pink pupae show a crop and proventriculus well developed (fig. 1E). The hypopharyngal glands also have increased in length. Sections from this phase show the proventriculus bulb already well differentiated (fig. 21) and the myoblasts starting elongate radially to the lumen. Only a few mitosis still occur in the epithelial cells lining the bulb lumen. The mitotic fuse is orientated in a way permits the bulb lips to project into the lumen.

In the red eyed pupa the morphology of the foregut (fig. 1F) is practically still the same. Histologically the proventriculus is already compltely differentiated, with the square shape and four lips of the proventriculus well delimilated (fig. 23). The passage into the ventriculus is still closed, but the primordium of the cardiac valve is already formed. In the beginning the partition between the fore and midgut (fig. 18) consists of two layers of cells (the ventricular and foregut epithelia). At the present stage both layers are perforated and the lateral walls of the future openning are formed (fig. 22). From the proventriculus side the tissue plug-cells are already distached from the proventricular neck walls. The cells that will constitute the valve are stomodeal in origin, according to Dobrovsky (1951).

The crop walls until the red eye stage remain unchanged, consisting of a rather smooth cubic epithelium and the usual muscular layers. But in the brown eyed pupae the crop walls appear formed by a squamous and very folded epithelium (fig. 24). Morphologically the crop also shows evidences of that transformation (fig. 16) because it appears larger, and with thinner walls. Another thing to notice in this stage is the hypopharingeal gland in which the secretory cells are already well dictached from the excretory canal given to it the aspect of a long cluster of small alveoli (fig. 1G). This is already the aspect that these organs take in adult. The proventricular bulb has also, by this time, its final form (fig. 25 and 28).

By the end of the pupal period, in tre brown yed pupae in which the body is beginning to became pigmented (fig. 1H), the foregut presents the imago aspect in all its length. The crop walls are so thin that the proventricular bulb can be seen by transparency. At this time the passage into the ventriculus is still closed, but it will open two or three days before emergency.

During the pupal stages the foregut greatly elongates and becomes differentiated into a pharynx, a eosophagus, a crop or honey stomach, and a proventriculus. The elongation takes place, first at the expense of cellular division that occurs between the white eye and pink eye stages and later by cells elongation and differentiation. In the adult the stomodaeum is four times longer then in the larva and 1,5 longer than in white eyed pupae.

The midgut — The larval midgut occupies most of the space in the body cavity. The whole food tract is adjusted to serve the larval function. The midgut attains the largest size of the digestive tube on account of storing all the food the larva can ingest during the short time available to growth. At the time of hatching the larval midgut yet contains yolk (fig. 26) but soon it is substituted by the food eaten by the larvae. As the larvae eat the ventriculus volume increases. By the end of the larval stage (fig. 1A) the ventriculus is a tubular bag occupying nearly two thirds of the space of the larval body.

The four types of cells recognized in the honey-bee midgut (Anglas, 1901; Nelson, 1924 and Oertel, 1930) are also found in the present case.

However, it seems that the so called collar cells consist really of two different types of cells. The first type was already mentioned and constitutes the imaginal ring (fig. 6). Following this, there is another set of four cells, very similar to the formers. They are narrow and tall cells whose inner border have not a distinct ending an protrude into the lumen of the midgut seeming to be continuous with the material forming the peritrophic membrane (fig. 27).

The digestive cells vary in aspect during the larval period with their location and also with the age increasing. In an early larva they form a cylindrical or cuboidal epithelium that lines almost all midgut and in whose inner surface is a distinct striated border (fig. 29). As soon as the larval food starts to enter the ventriculus many small granules and globules (fig. 29) appear in the apical border of these cells. In a larva two days old the digestive cells show bulbles (zeiosis) production in their apical borders (fig. 30 and 31). The bubbling of the apical membranes of the digestive cells continues during the rest of the larval life and probably is the way by which the secretion is released.

The generative cells (fig. 32) are very small and inconspicuous, most of the times difficult to see. They are nestled in the basis of the digestive cells, attached to the basement membrane. In Melipona they are few in number forming groups of 3 or 4 cells.

The barrier which closes the passage from midgut into hindgut is disrupted at the end of the larval period, by the only time the larval intestine empty. In young larvae the septu mbetween ventriculus and proctodaeum is a double wall made by the mid and hindgut epithelium (fig. 33). The ventricular cells have here a special type, smaller and taller than the digestive cells and vacuolated in the inner pole. Later a plug of these cells growth backward (fig. 34) forcing the hindgut epithelium. By the end of the larval stage (larvae 6 days old) the hindgut epithelium breakes and the midgut walls extend a little into the proctodaeum forming the pyloric valve. The way in which this barrier is disrupted is similar to that of the cardiac valve in old pupae.

The muscular fibers in the larval midgut are very thin and incospicuous but at least two layers can be distinguished: the inner circular and the outer longitudinal.

The ventriculus is the most changeable part of the digestive tract because of its role in digestion. During the larval life some modifications can be detected in it but the most striking change in its external appearance is the collapsing of its walls. After the discharge of the faces the ventriculus appears as a flattened tube almost the same diameter as fore and hindgut (fig. 18). This change occurs at the end of larval life as well, as the replacement of the old epithelium by a special pupal one (Lotmar, 1943).

The large digestive cells of the larva became vacuolate and degenerate being casted of (fig. 36) and thrown into the lumen through where they will be digest or eliminated. Actually, in spite of, this breaking down of the epithelial cells, the ventriculus always has a wall (fig. 35), because the basement membrane and generative cells are always present.

The morphological aspect of the midgut during the prepupae stage is show in figure 1B. During the prepupal stages the degenerated larval epithelium is replaced by a new epitelium suitable to the pupae. The generative cells (fig. 36) are supposed to replace the larval epithelium but cellular divisions were not seen in this cells in any phase of the gut development. According to Dobrovsky (1951) the replacement starts anteriorly and proceeds posterior. In the prepupae the ventriculus shows the aspect of figure 38. The reason for the formation of a temporary pupal epithelium is not very well understood, since the pupa do not eat.

Soon the midgut expands again and takes the form of a bottle (fig. 10), larger in diameter posteriorly. At this time (white eyed pupae) the ventricular epithelium appears as showed in the figure 39.

The expanding of the ventricular diameter proceeds anteriorly (fig. 1D) while the epithelial cells increase in height (fig. 37).

In the pink eyed pupae the diameter of the ventriculus is again uniform (fig. 1E). At this time another transformation starts in the midgut. The top of the epithelial cells are casted of (fig. 40) and thrown into the lumen. This casting off also starts anteriorly (and in some specimens it can be seen in the cardiac valve region, as soon as, the white eyed pupae fig. 18) and proceeds posteriorly. In spite of the epithelium lost its apical border the cells nuclei appear very healthy, i. e, they to not show picnotic degeneration (fig. 41).

The ventriculus in the red eyed pupae have increased in length (fig. 1F). The epithelial cells, now appear low and irregular in shape (fig. 42). In the next stage, the annulations in the midgut wall are already visibles (fig. 1G) and correspondently the epithelium starts to take the adult aspect (figs. 43 and 44) which is seen in figure 46.

The muscular wall is not reorganized during prepupation, but it will be during the last metamorphosis in the midgut (bright pink eyed pupae to red eyed pupae), which will produce the adult epithelium.

During the ventricular metamorphosis it is surprizing that the role of the generative cells could not be well interpreted because, in spite of being more numerous in prepupae and pupae we never saw them dividing.

The metamorphosis of all digestive tract begins in the ventriculus, in the last hours of larval life and rapidly transform the simple food tract of larval into the diversified alimentary canal of the adult. The ventriculus also increases in length during these changes. From the last larval stage to the last pupal stage it increases 2 times, but from larvae to prepupae it shortens about 20%, so the ventriculus of the white eyed pupae is less than half of the larval ventriculus in length.

The peritrophic membrane — A peritrophic membrane lines the epithelium of the larval midgut. Nelson (1924) described this membrane as a very thick homogeneous layer of apparently gelatinous consistency. This membrane is clearly seen to arise from a ring posterior to the imaginal ring (fig. 29). Nelson (1924) and Evenius (1926) atributed the same origin to the peritrophic membrane of **Apis** with the difference that they thougth that the imaginal cells gave origin to it.

According to Kusmenko (1940), another peritrophic membrane is present in the larvae. This one, formed by the general surface of the ventriculus. We could not detect this second membrane in **Melipona's** larvae, but in figure 29, a more colored band can be seen in the striated border, maybe the material of a future peritrophic membrane.

The larval peritrophic membrane is eliminated with the faces at the end of larval life. According to Dobrovsky (1951), in **Apis,** no other peritrophic membrane is formed until the end of the pupal period, but Hering (1939) said thats the peritrophic membrane is formed in 5 stages beginning in 18th day of development, i. e, at the beginning of pupation. In **Melipona,** after the larval intestine discharge, a peritrophic membrane was not seen until the brown eyed pupa.

The ring cells around the base of the stomodeal valve degenerates in adults. They appear as flattened cells connecting the valve with the ventriculus cells (Cruz-Landim and Rodrigues, 1967). Nothing similar to the activity they display in larva is seen in adult, therefore, the peritrophic membrane in **Melipona's** adults is formed by the ventriculus regular epithelium. Snodgrass (1956) also thinks that there is no doubt that the adult peritrophic membrane in **Apis** is consecutively formed from the entire length of the midgut epithelium.

The hindgut — The hindgut of a larva is a slightly flattened tube, relatively long (fig. 1A) and shaped like an S. The epithelial cells linning the hindgut are thin colunnar cells (fig. 46). A cuticle, continuous with that of the body wall, lines the epithelium internally. The muscular wall is heavier than in the foreparts of the gut and consists of circular fibers. In a larva 3-4 days old the hindgut is already histologically differentiated in a anterior and posterior portion (fig. 47). Dobrovsky (1915) mentioned the appearance of pad like structures in the hindgut of late larvae. In the present case, it was verified the occurrence of papillae like folds (figs. 47 and 48) but, it is difficult to say if it is a rectal-papillae or a regular intestinal fold. Adults of some primiitve bees, instead of rectal pads present rectal papillae (Ferreira and Cruz-Landim, 1969). The rectal papillae are simply folds of the rectal epithelium very similar to what is shown in figure 48. They present an outer pore whereby penetrate tracheoles. Snodgrass (1956) consider this types of rectal organ, more primitive than the regular rectal pads. It can be admited that the **Melipona**'s larvae present more primitive structures than the adult.

The communication between mid and hindgut remains closed during all larval life, being opened just once before prepupation. A pyloric valve is formed by this time. After prepupation this passage is closed again by a tissue plug dirived from the adjacent epithelial cells of the ventriculus (Oertel, 1930). During prepupation the proctodaeum becomes a straight tube (fig. 1B). In this phase many mitosis occur mainly in the anterior part (fig. 49). The mitosis lead to increase of the hindgut in length, so in the white eyed pupae it forms a loop (fig. 1C). In this phase there is no differenciation along all length, being the diameter the same from the anus to the pylorus the same occurring with histological organization (fig. 50).

The development of the rectum and the rectal pads or glands (fig. 51 to 56), proceeds simultaneously. The bright pink eyed pupa presents a slightly enlargement in the posterior end of the hindgut (fig. 1D). In this case it can already be distinguished a small intestine and a rectum. The rectum increases rapidly in length and in diameter (fig. 1E) in such a way that in the pink eyed pupae it has almost the size of the last pupal stage. The hindgut is completly differentiated at the end of the eye pigmentation (brown eyed pupae). At this time the small intestine shows its adult aspect (fig. 56) with six longitudinal folds along its length and rectum walls very thin, and folded, constituted by squamous cells (fig. 57) similar to the crop walls. The hindgut greatly increase in length during the pupation. This increasing is about 2.5 times.

The Rectal Pads — It was impossible in our preparations to detect the rectal pads thickennings before the brigth pink eyed pupae, and at this time they appear as shown in figure 52, a row of tall cilyndric cells, with the nuclei in the apical pole, and in whose basis were many small corpuscules. The chromatic material from both, the small corpuscules (whose cellular condition is not very clear) and epithelial cells stains heavy by the hematoxylin. In the next phase, the aspect is essentialy the same, but in the photo corresponding to this stage (fig. 53) it can be seen the above mentioned small corpuscules inside the epithelium. Until now the limit between the epithelial cells and the globules in their basis are not sharp, but in the next stage it is (fig. 54). Also the corpuscules clearly show now their cellular condition because a cytoplasm mass can be seen around the nuclei. In this phase (red eyed pupae), there are a tendency of these cells to organize in three rows, from which the outer is continuous around all rectum periphery.

The rectal pads in adult **Melipona** are six oval organs located in the anterior part of the rectum forming two alternate rows (Cruz-Landim and Rodrigues, 1967). They are hollow organs constitued by a inner layer of tall cells and two outer layers of flat cells, which encircle the hollow. In the brown eyed pupae the rectal pads present already its definitive shape (fig. 55).

Evenius (1933) and Lotmar (1945) claimed that the two outer layers of cells are formed by adventitious mesoderm from the body cavity, which acumulates around the rectum and give origin to outer cells of the pads and also to the muscular sheaths. Dobrovsky (1951) postulated a completely differentiation origin. He thougth that the outer cells comes from irregular division from the epithelial cells as the so called chromatic bodies of many authors.

Unfortunatelly it was not possible to refuse or confirm one of the above theories. The similarity between the outer cells and the myoblast that surround the rectum seem to indicate that the Evenius-Lotmar theory is right, but in another hand, the presence of "chromatic bodies" inside the epithelium reinforce Dobrovsky's theory. Ferreira and Cruz-Landim (1969) found certain primitive bees from whose the rectal pads are absent. In this case an acummulation of mesodermal cells appear around the rectum, but even in this case is difficult to say if it was or not a coincidence.

The Malpighian Tubes — In **Melipona**'s larvae there are eigth Malpighian tubes. They are long extending anteriorly from the point of union of the mid and hindgut, reaching nearly the thorax. During the larval life the Malpighian tubules acumulate excreta and become very dilated with them (fig. 58). About 6 days after hatching the Malpighian tubules empty their content into the hindgut (at the same time that the ventriculus does) and begin to degenerate. As pointed out by Dobrovsky (1951) in **Apis**, the larval rectal papillae of **Melipona** appear just before the emptying of the Malpighian tubes and ventriculus what sugest some function of this structure connected with the excretion.

In early prepupa the larval and adult Malpighian tubes coexist. The degeneration of the larval tubes is about completion by the time of the end of the prepupation (fig. 59). The new Malpighian tubes originates from imaginal buds in the pyloric region of the hindgut. Its development starts yet in the larvae. The figure 60 shows the mid-hindgut contact from a larvae 6 days old. This larva shows the beginning of the adult Malpighian tubes development. In this photo it can be clearly seen that the Malpighian tube is connected with the hindgut. In spite of the Malpighian tubes epithelium resembles the midgut epithelium (striated border) it is ectodermal in origin contrarily what thought Trappamann (1923) and Weil (1935). The Malpighian tubes do not suffer transformations during the pupae life. They present during pupation the aspect of figure 61, they only increase in length by cellular division at the hindgut contact. After pink eyed pupae the divisions stop so they have alread reached the complete development.

At the end of the pupae stage we have the simple digestive canal of the larvae changed into the diversified alimentary tract of the adult.

In the pupation beginning most of the digestive trat is located in the thorax (fig. 1). As the development proceeds and the foregut elongates is a progressive migration backwards. At the end of the eye pigmentation the ventriculus. proventriculus and crop are already located in the abdomen.

CONCLUSIONS

The post-embryonic changes of the digestive tract of **Melipona quadrifasciata anthidioides** take place in larvae, prepupae and pupae (Table 1).

The larval transformations refer to mid and hindgut. The foregut remains unchanged during all larval life.

When the larval ventriculus is emptyed of its yolk contents and the larvae starts to feed the ventriculus epithelium changes from a aparently inative epithelium to one actively secretory (2 days ald larvae). But the first structural change occur in the hindgut with the appearance of the larval rectal papilae (5 days old larvae). We are not two sure about the nature of those rectal folds, if we have not read the Dobrovsky paper it would pass unnoticed, but the fact of some adult bees present rectal papilae very similar to that is a point to consider. The second structural change occurs by the end of the larval period and consist of the dissolution of the tissue plug between the mid and foregut (6 days old larvae). The ventriculus and the Malpighian tubes are than emptied, and consequently they collapse and degenerate. The reorganization of both begin yet in the larvae.

During the prepupation the degenerated larval epithelium of ventriculus is superseded by a pupae epithelium. The role of the generative cells in this substitution is not quite clear. Mitotic divisions begin in the posterior part of the foregut and anterior part of the hindgut.

The greatest changes occur during the pupation. The foregut differentiates in a pharynx, oesophagus, crop and proventriculus. The foregut differentiation is completed by the time in which the body pigmentations starts. The midgut has its epithelium substituted again by a imaginal or adult epithelium. The replacement starts anteriorly with the beginning of the eye pigmentation and is finished in the brown eyed pupae. The hindgut differentiates into intestine and a rectum. The rectal pads appear as early as white eyed pupae and have the adult fashion in the brown eyed pupae. The Malpighian tubes are eight in the larvae and 40 in the adult. In red eyed pupae they have already the adult size.

The metamorphosis of the ectodermal and mesodermal parts of the alimentary canal proceeds differently. In spite of the changes, in the fore and hindgut were more stricking they are gradual and without acentuated degeneration process. In the foregut some degeneration occurs in the eosophagus, but in the hindgut it was not seen. The cellular divisions are numerous in these two parts; they provide their elongation and differentiation. The ventriculus has its epithelium replaced twice and also increases in length, mainly during the red eye stage, but divisions were not seen. The elongation of the ventriculus occurs by the time of the last epithelial substituition.

We can say that the post-embryonic changes in **Melipona** digestive tract begin 2 days after the egg hatching and finish 6 days before emergence. In brown eyed pupae the digestive tube is already very similar to the adult.

Some points remain obscure in our work. 1) The role of the generative cells. They do increase in number and size after the ventricular epithelium degeneration proceding the replacement, but divisions on them were never seen. 2) The origin of the rectal pads outer layers was not also clarified.

Ours findings do not differ from those written by other authors about **Apis** but even Dobrovsky who did the most complete paper in the subject passed over proventriculus differentiation.

The special ring of cells secreting the larval preritrophic membrane is discribed for the first time.

LITERATURE

Anglas, J. — 1901 — Observations sur les métamorphosis internes de la guêpe et de l'abeille. Bull. Sci. France et Belgique, **34**: 363-473.

Bordas, M. L. — 1895 — Tube digestif des Hyménoptéras Anim. Sc. Nat. Zool. **19**: 197-358.

Cruz-Landim, C. — 1966 — Alguns dados sôbre o desenvolvimento de **Melipona** (Hym. Apoidea). Rev. Brasil. Biol., **26** (2): 165-174.

Cruz-Landim C. and L. Rodrigues — 1967 — Comparative Anatomy and Histology of the Alimentary Canal of Adult Apinae. J. Apicultural Res., **6** (1): 17-28.

Cruz-Landim, C. and M. L. S. Mello — 1968 — The post-embryonic Changes in **Melipona quadrifasciata anthidioides** Lep. (Hym. Apoidea) II. Development of the salivary glands system. J. Morph., **123**: 481-502.

Day, M. F., and D. F. Waterhouse — 1953 — Functions of the alimentary system. In Roeder, Insect Physiology, **II**, 298-310.

Dehn, Madeleine von — 1933 — Untersunchungen über die Bildung der peritrophischen Membrane bei den Insekten Zeitschr. Zellfirsch mikr. Ant. **19**: 79-105.

Dobrovsky. T. M. — 1951 — Postembryonic changes in the digestive tract of the worker honeybee (**Apis mellifera** L.). Cornell. Univ. Agr. Exp. Stat., Mem. **301**: 1-45.

Evenius, C. — 1926 — Der Verschiuss zwischen Vorder-und Mitteldarm bei der postembryonalen von **Apis mellifera** L. Zool. An. **68**: 249-262.

Evenius, C. — 1933 — Über die Entwicklung der Rektaldrüsen von **Vespa vulgaris**. Zool. Jalb., Anat. **56**: 349-372.

Ferreira, A. and C. Cruz-Landim — 1969 — Comparative Studies on the "Rectal Glands" of Apoidea (Hym.). An. Acad. Brasil. Ciências, no prelo.

Green, T. L. — 1933 — Some Aspects of the Metamorphosis of the Alimentary System in the Wasp, **Vespa vulgaris** (Hymenoptera). Zool. Soc. London: 629-644.

Henson, H. — 1933 — The Development of the Alimentary Canal in **Pieris brassicae** and the Endodermal origin of the Malpighian Tubules of Insects. Quart. J. Microscop. Sci. **75**: 283-305.

Hering M. — 1939 — Die peritrophischen Hüllen der Honigbiene mit besonderer Berüchtegung der zeit während der Entwicklung des imaginalem Darmes. Ein Beitrag zum Studium der peritrophischen Membran der Insekten. Zool. Jahrb. Anat. **66**: 129-190.

Kusmenko, S. — 1940 — Über die postembryonale Entwicklung des Darmes der Hönigbiene und die Herkunft der larvalen peritrophischen Hüllen. Zool. Jahrb., **66**: 463-530.

Kusmenko, S. — 1941 — Herkunft der Malpighian Gefässe der Hönigbiene. Zool. Jahrb. Anat., **67**: 271-292.

Lotmar, R. — 1945 — Die Metamorphose des Bienendarm (**Apis mellifera**). Beigheft Schweiz Bienenzeitung, **1** (10): 443-506.

Nelson, J. A. — 1915 — The Embryology of the Honey Bee, 282 pp Princeton.

Nelson, J. A. — 1924 — Morphology of the honeybee larva. J. Agr Res., **28** (12): 1167-1273.

Oertel, E. — 1930 — Metamorphosis in the honey bee. J. Morphol. **50**: 295-340.

Sakagami, S. F. and R. Zucchi — 1966 — Estudo cimparativo do comportamento de várias espécies de abelhas sem ferrão com especial referência ao processo de aprovisionamento e postura das células. Ciência e Cultura, **18** (3): 283-296.

Shmidt, von G. H. — 1964 — Histologische Untersunchungen zur Metamorphose des Mitteldarmepithels von **Formica polyctena** Foerst (Ins. Hym.). Biol. Zentr. **83** (6): 7171-724.

Schnetter, M. — 1934 — Morphologische untersuchungen über das differenziernzentrum in der Embryonalentwicklung der Hönigbiene, z. f. Morphol. Okol. Tiere, **29**: 114-195.

Snodgrass, R. E. — 1925 — Anatomy and Physiology of the Honeybee. 327 pp., New York and London.

Snodgrass R. E. — 1956 — Anatomy of the Honey Bee. 334 pp. Comstock Publ., Ithaca, New York.

Trappman, W. — 1923 — Die Malpigisch Gefässe, Anatomie und Physiilogie des Zwischendarms, Die Bilding peritrophischen Membran Die Retaldrüsen von **Apis mellifera** L. Arch. Bienenkunde, **5**: 177-220.

Weill, E. — 1935 — Vergleichenden-Morpholigische Untersunhungen a einiger Apidem und Vespiden. Zeitschr. Morph. Okol. Tiere. **30**: 438-478.

TABLE I

Pist-embryonic changes in **Melipona quadrifasciata anthidioides** Lep.

Dev[illegible]opment Stage	Foregut	Midgut	Hindgut	Malpighian Tubes	Rectal pads
Larvae 2 days old		Secretory cells in ventriculus			
Larvae 5 days old			Rectal papillae-like structures		
Larvae 6 days old		Feces elimination. Collapse	Opening of the passage between mid hindgut	Excreta elimination. Collapse	
Larvae 7 days old		Epithelial degeneration		Degeneration of the larval tubes and activation of imaginal buds	
Prepupation	Eosophagus degeneration. Cellular divisions in the posterior part	Replacement of the larval by pupal epithelium	Cellular divisions in the anterior part	Elongation of the adult tubes	
White eyed pupa	Crop differentiation		Elongation	Elongation	
Bright pink eyed pupa	Hypopharingeal buds proventriculus differentiation	Beginning of the pupal epithelium degeneration	Rectum differentiation	Elongation	Rectal pads thickenings
Pink eyed pupa	Proventriculus differentiation	Pupal epithelium degeneration	Rectum differentiation	Elongation	Rectal pads differentiation
Red eyed pupa	Proventriculus differentiation	Epithelial replacement, ventriculus elongation	Rectum differentiation	End of elongation	Rectal pads differentiation
Brown eyed pupae	Crop wall consisting of flat cells	External annelations visible	Rectum in the adult fashion	Adult aspect	Differentiation completed
Beginning of body pigmentation	Adult aspect	Adult aspect	Adult aspect	Adult aspect	Adult aspect

ABBREVIATIONS

A — annus
AMT — adult Malpighian tubes
B — brain
BW — body wall
c — cuticle
cc — casted cells
cg — chromatic globule
Chrb — chromatic body
cm — circular muscles
cp — cardiac plug
Cr — crop
Cr ep — crop epithelium
C.V — cardiac valve
dc — digestive cells
ds — dorsal side
E ep — epipharynx epithelium
em — extrinsic muscles
ep — epithelium
FB — fat body
Fc — fat cell
gc — generative cells
h — hollow
HG — hind gut
IC — imaginal cells
il — inner epithelial layer
IR — imaginal ring
irc — imaginal ring cells
lep — larval epithelium
lm — longitudinal muscles
LMT — larval Malpighian tubes
M — mouth
m — muscles
MT — Malpighian tubes
my — myoblast
n — nucleus
NG — nervous ganglion
Oe — oesophagus
oe m — oesophageal muscles
oen — oenocytes
ol — outer epithelial layer
p ep — pupae epithelium
Ph — pharynx
PM — peritrophic membrane
pmc — peritrophic membrane cells
pp — pyloric plug
P.S — pyloric septum
PV — proventriculus
PVb — proventriculus bulb
PVn — proventriculus neck
R — Rectum
Rep — Rectum epithelium
Rp — rectal papilla
Rpa — rectal pad
SG — silk glands
SI — small intestine
stb — striated border
tm — transverse muscles
V — ventriculus
Vep — ventricular epithelium
VS — ventral side
y — yolk

FIGURES

Fig. 1 — Schematic representation of the digestive canal development: A — larvae; B — prepupae; C — white eyed pupae; D — bright pink eyed pupae; E — pink eyed pupae; F — red eyed pupae; G — Brown eyed pupae; S — Brown eyed pupae with body pigmentation. **a.** marks the anterior end, and **d** the posterior end. **b** marks the occipital foramen localization and **c** the thoraxic abdominal passage. MT = Malpighian tubes; SG = silk glands

Fig. 2 — Larval foregut. 50x

Fig. 3 — Epipharingeal groove. 120x

Fig. 4 — Larval oesophagus. 200x

Fig. 5 — Cardiac Valve. 120x

Fig. 6 — Imaginal ring. 200x

Fig. 7 — Prepupae oesophagus. 200x

Fig. 8 to 16 — Cross sections of the fore — midgut transition. 8 = ventriculus; 9, 10, 11 e 12 = cardiac valve zone; 13, 14, 15 = proventriculus neck; 16 = proventriculus bulb. 200x

Fig. 17 — Hypopharyngeal glands buds. 120x

Fig. 18 — Proventriculus. The arrow points to mitotic cells in the neck zone. 50x

Fig. 19 — Mitotic divisions in the proventriculus. 500x

Fig. 20 — Proventriculus bulb. Note the mitosis (arrows) in the epithelium and the myoblasts elongation. 120x

Fig. 21 — Proventriculus differentiations. 50x

Fig. 22 — Zone of the cardiac valve. The arrows shows the point where the passage into the midgut will open. 200x

Fig. 23 — Proventriculus bulb in red eyed pupae. 120x
Fig. 24 — Crop in brown eyed pupae. 120x
Fig. 25 — Proventriculus bulb of brown eyed pupae. 120x
Fig. 26 — Ventriculus epithelium of young larvae. 200x
Fig. 27 — Differentiation of the imaginal ring. 500x
Fig. 28 — Proventriculus bulb of the adult. 120x
Fig. 29 — Ventriculus epithelium in early feeding larvae. 500x
Fig. 30 — Bubling of the larval epithelium. 200x
Fig. 31 — Bubling of the larval epithelium. 500x
Fig. 32 — Generative cells of the larvae. 120x
Fig. 33 — Mid-hindgut passage. 120x
Fig. 34 — Opening of the hindgut plug. 200x
Fig. 35 — Ventricular epithelium of a old larvae, preceding prepupation. 200x
Fig. 36 — Degeneration of the ventricular epitheliu min old

Fig. A-H

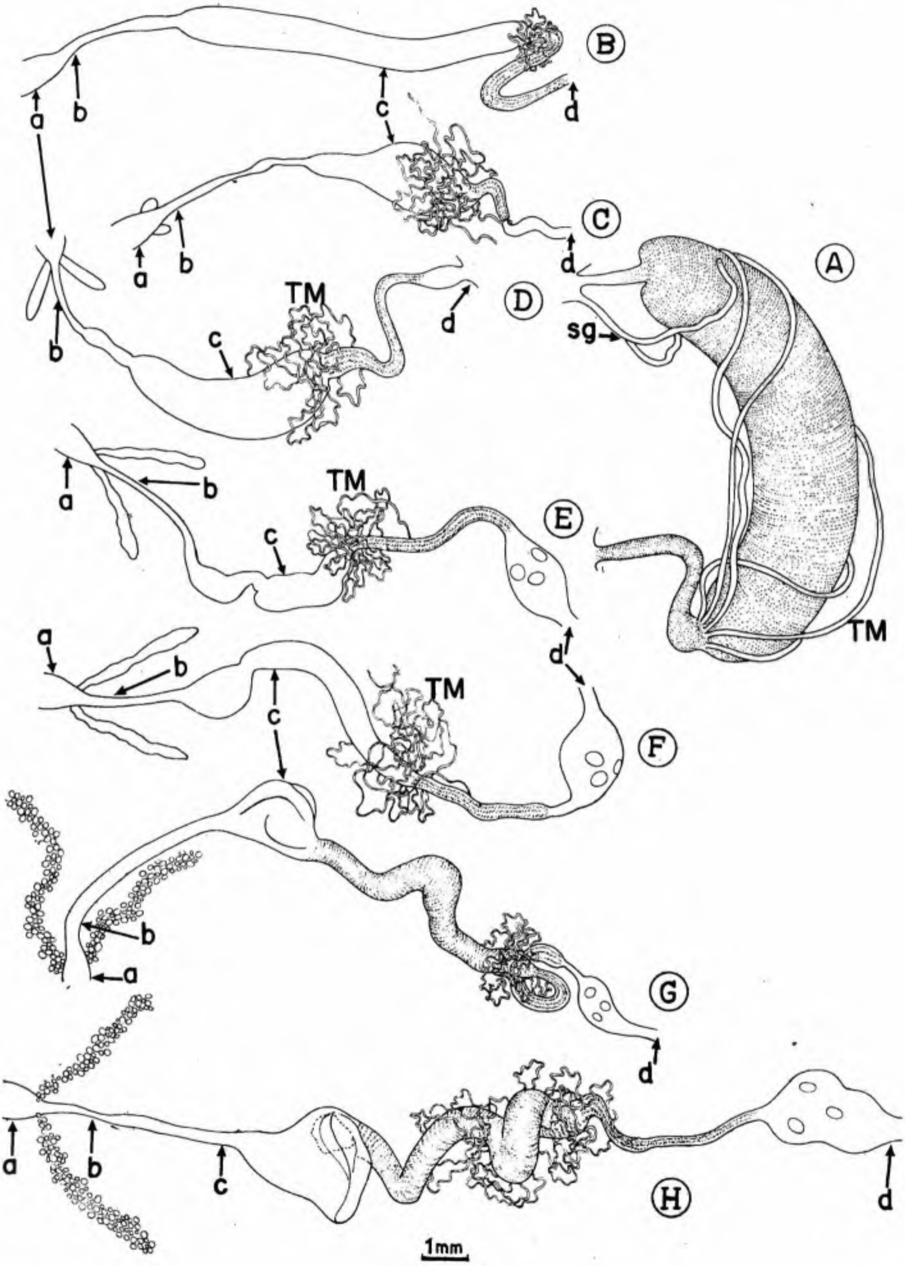
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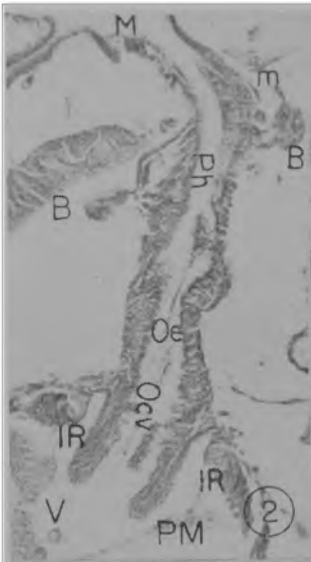
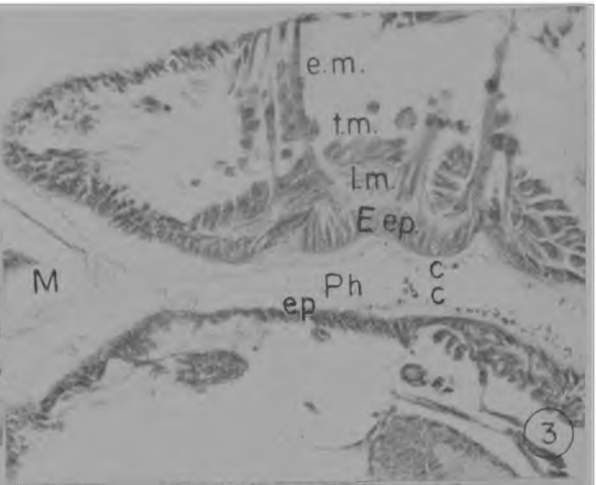
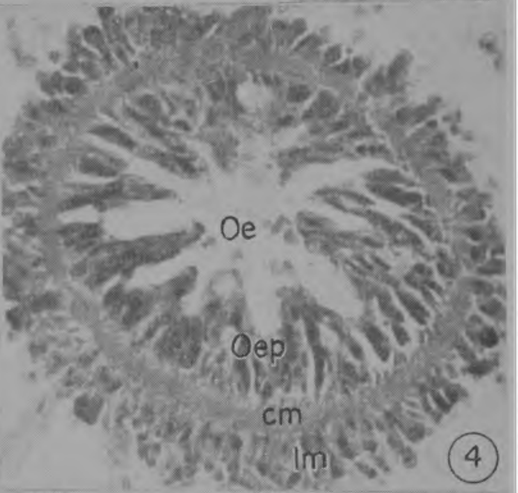
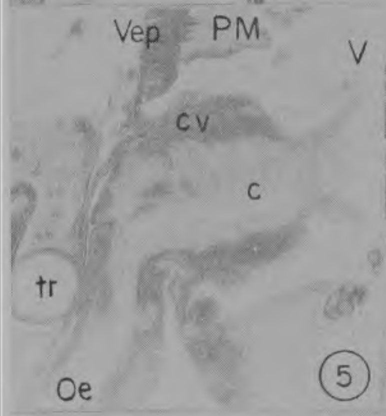
Plate 2
Figs. 1-7









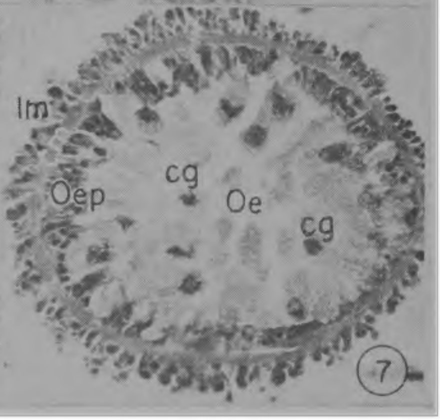
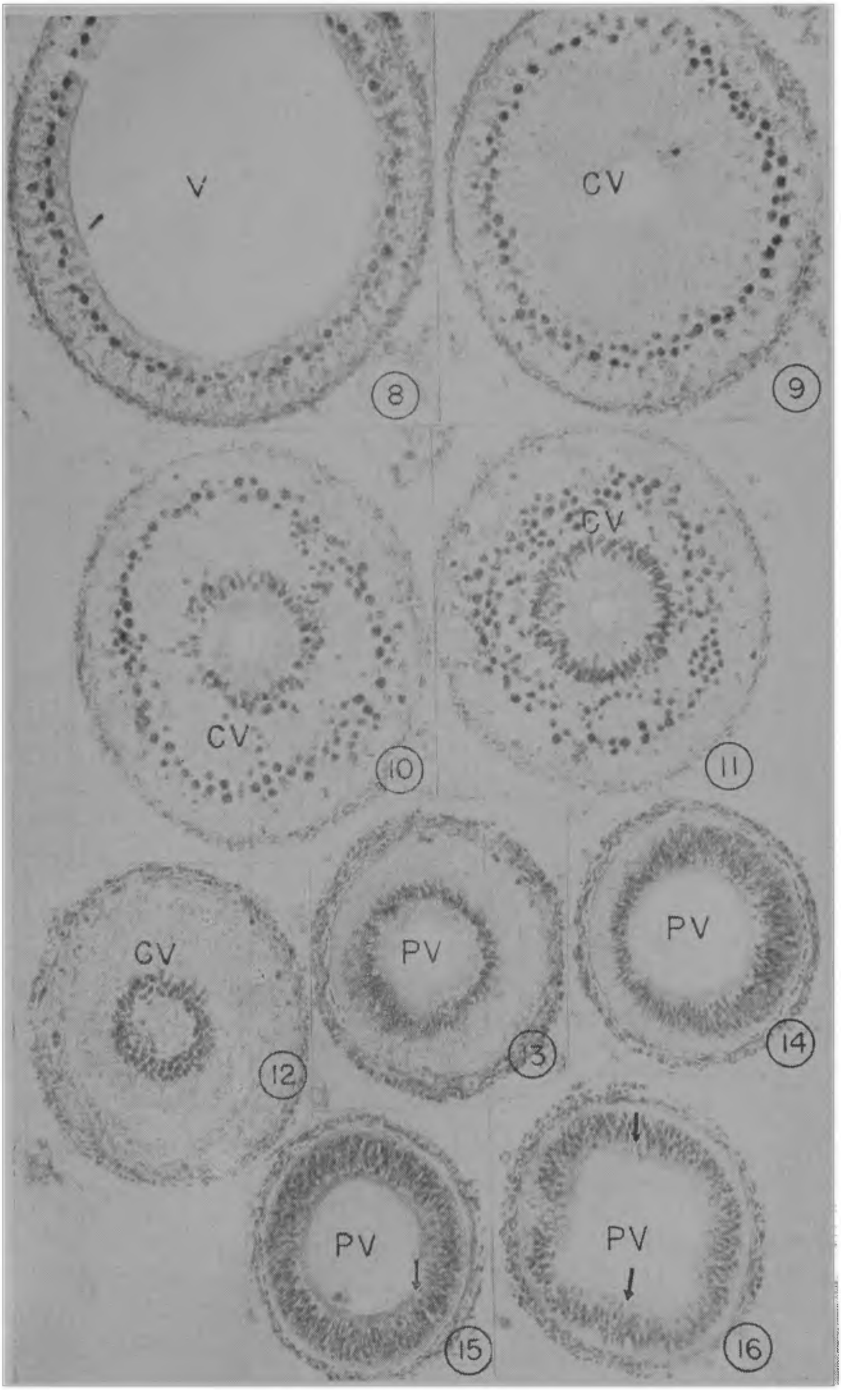
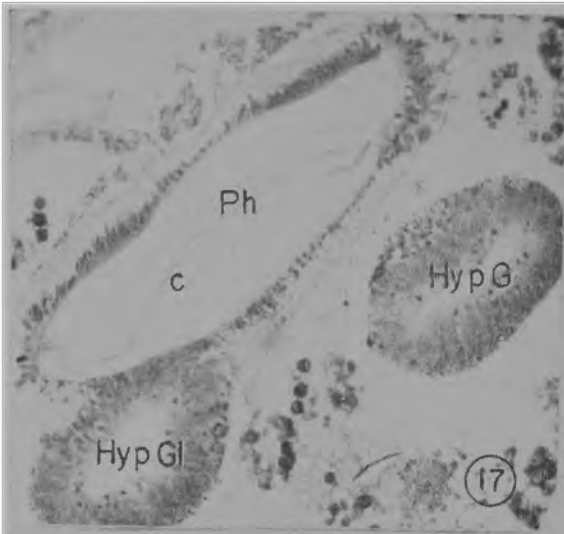


Plate 3
Figs. 8-16

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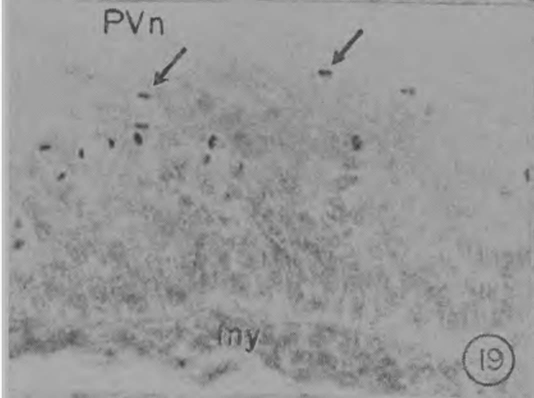
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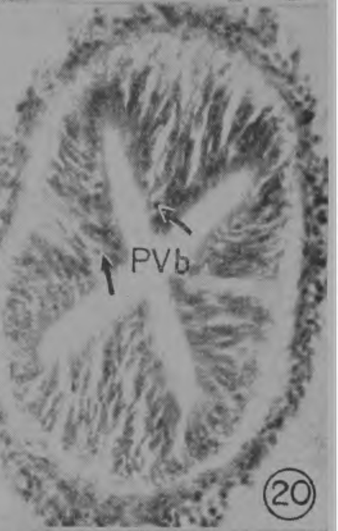
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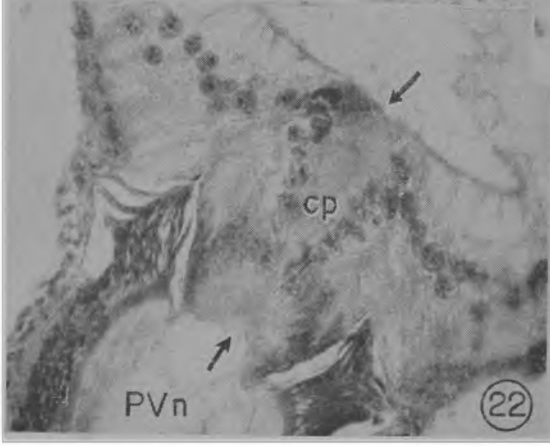
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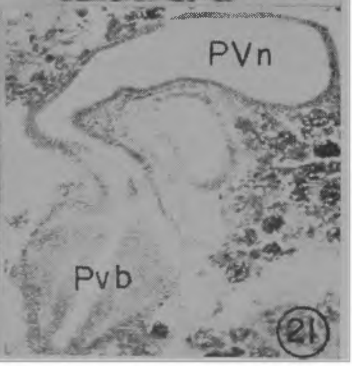
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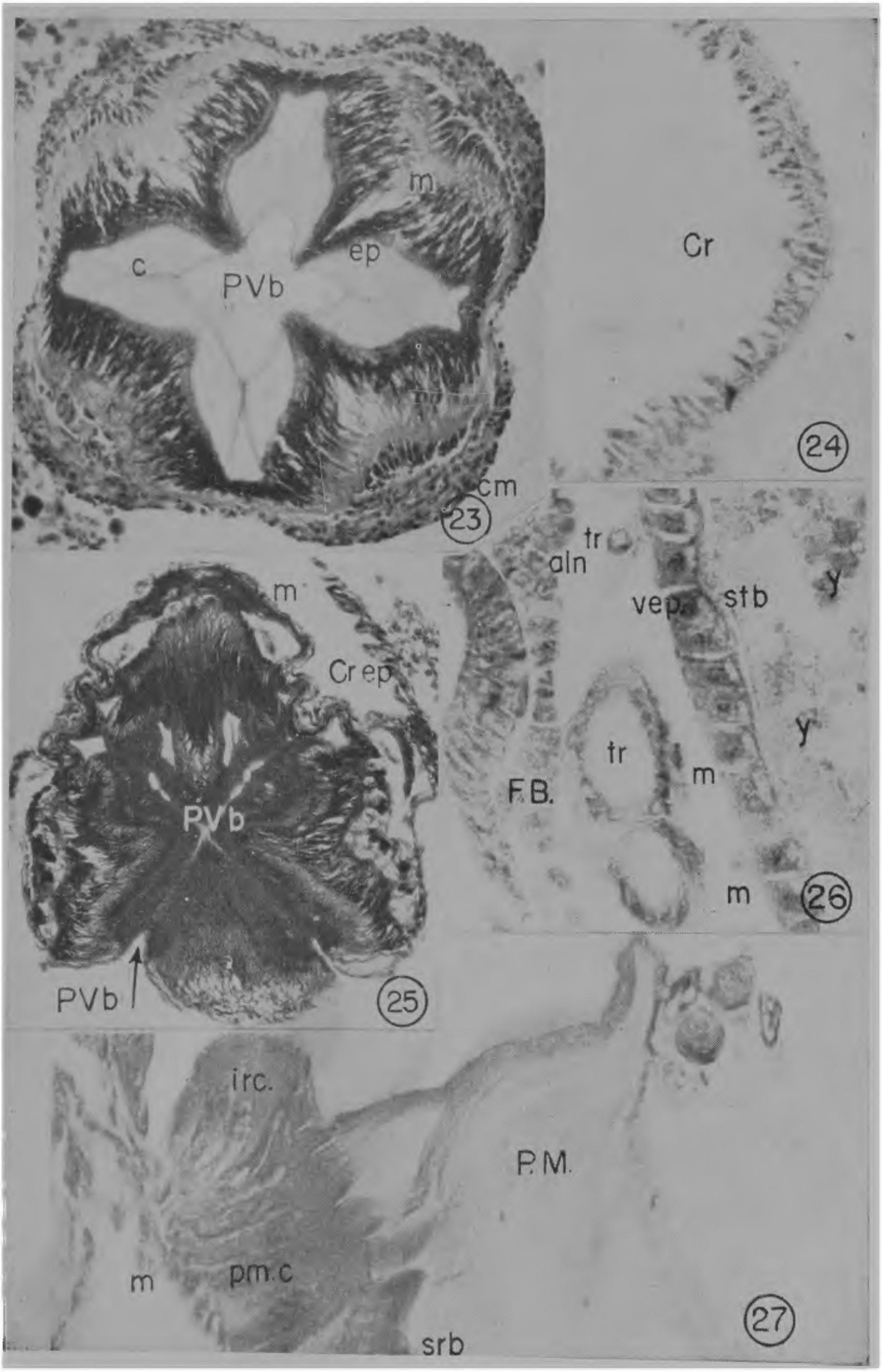
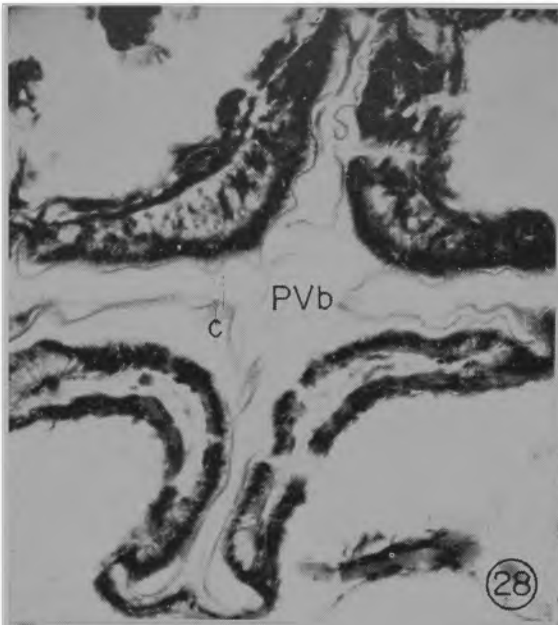
Plate 5
Figs. 23-27

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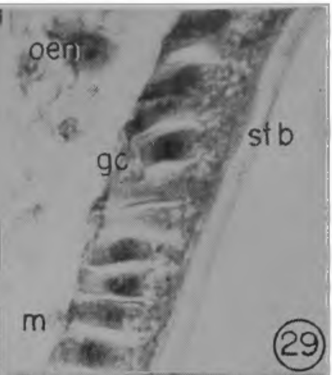
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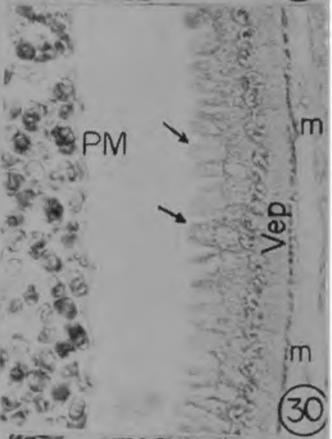
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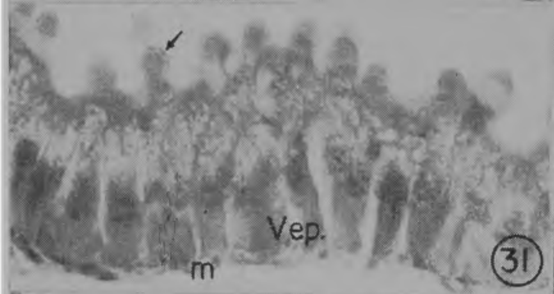
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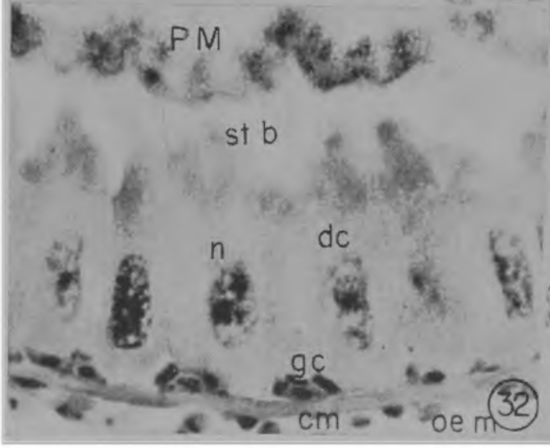
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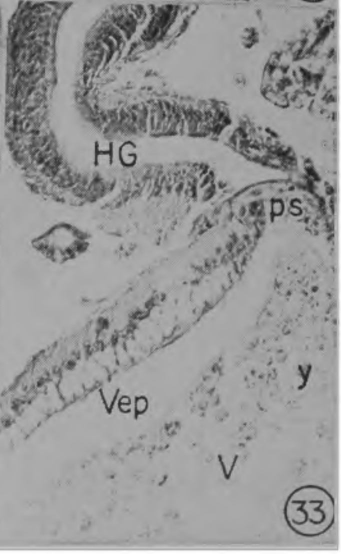
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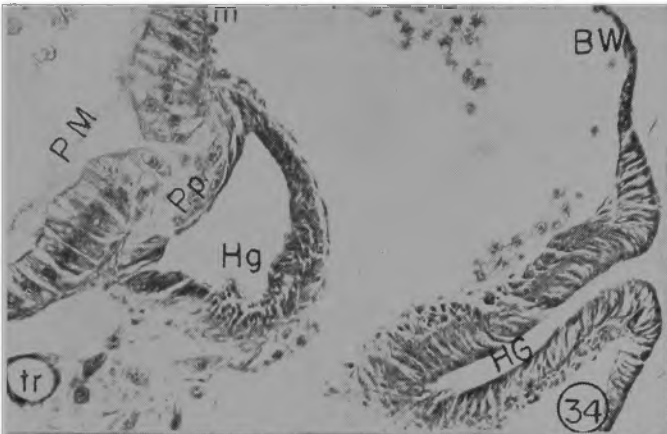
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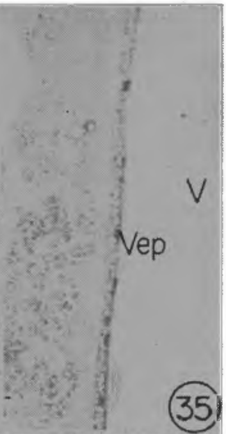
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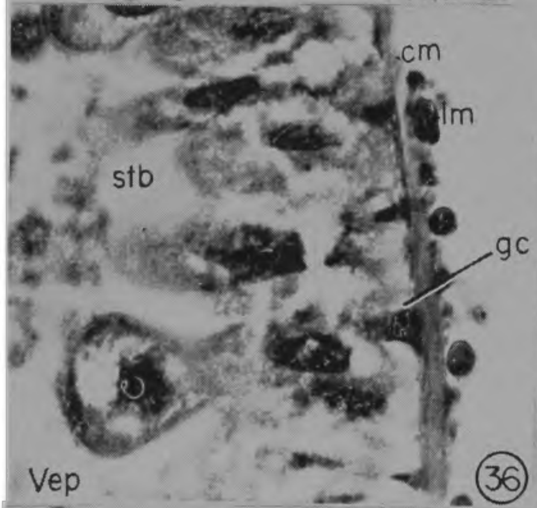
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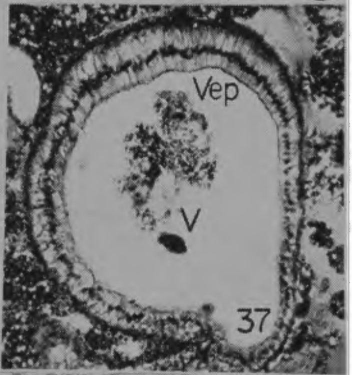
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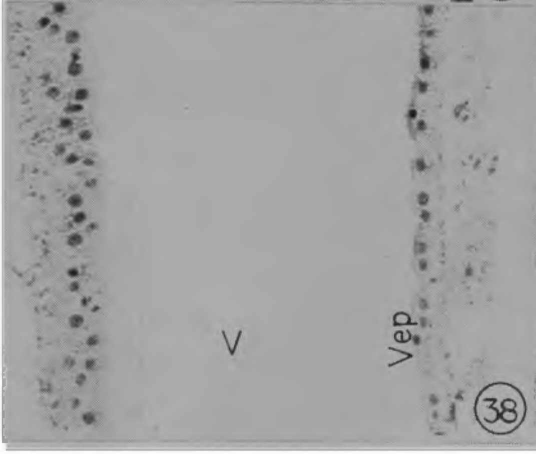
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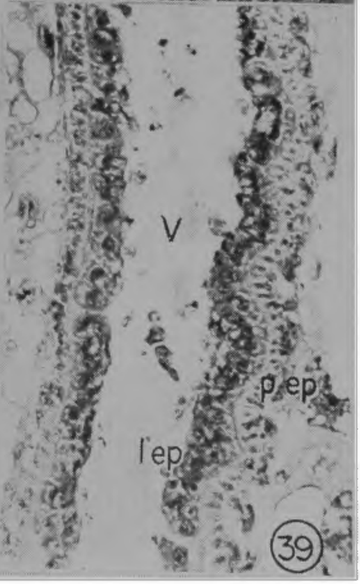
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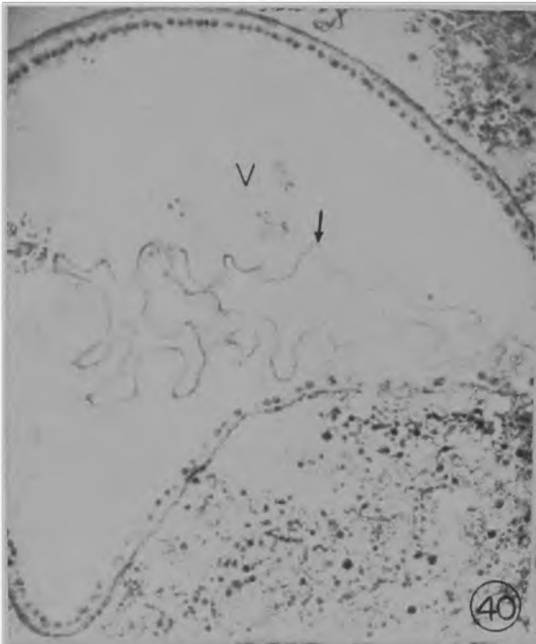
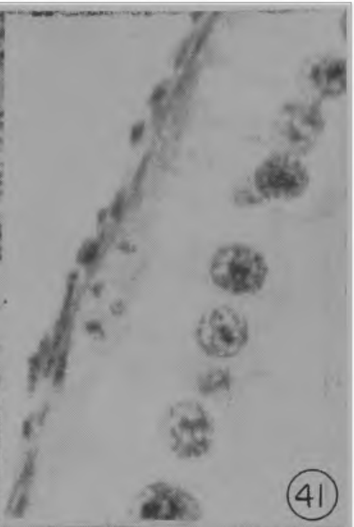
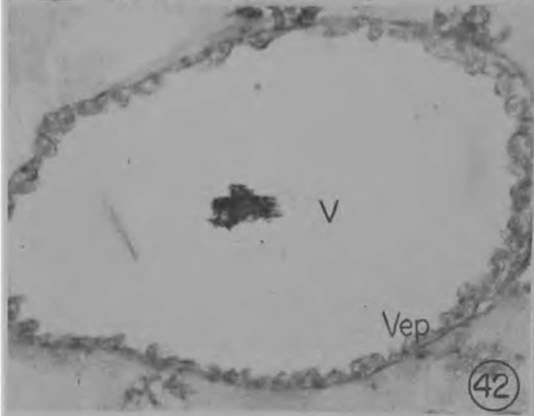
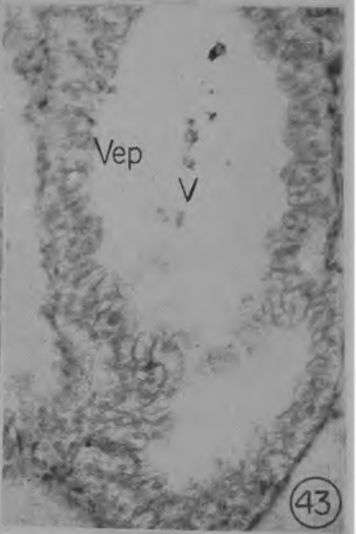
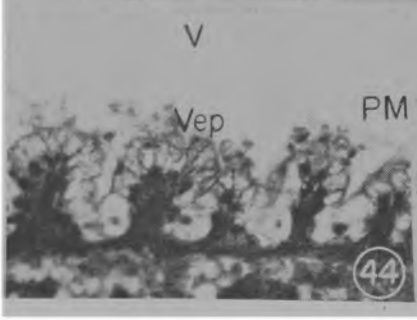
Plate 8
Figs. 40-45











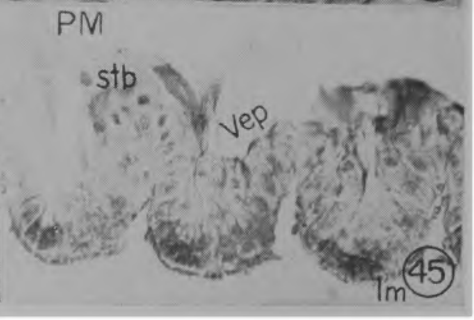
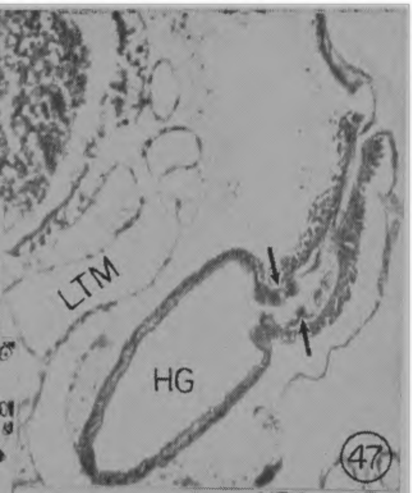
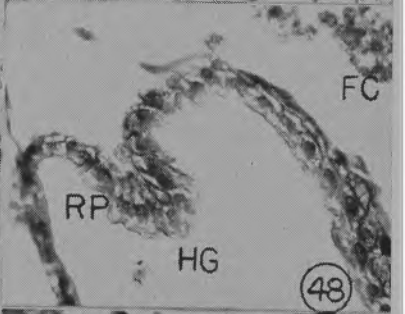
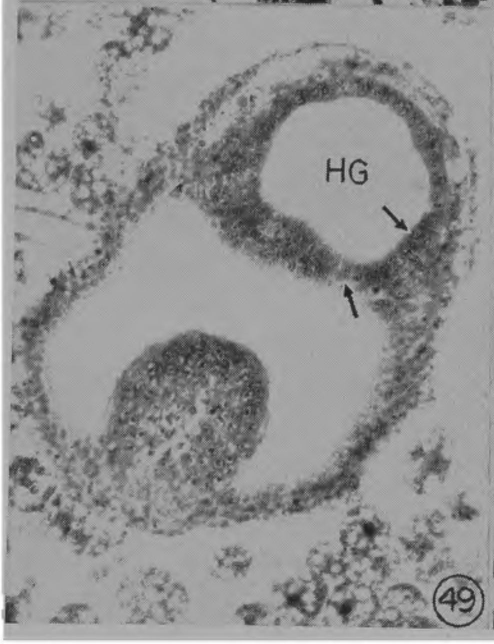


Plate 10
Figs. 46-50







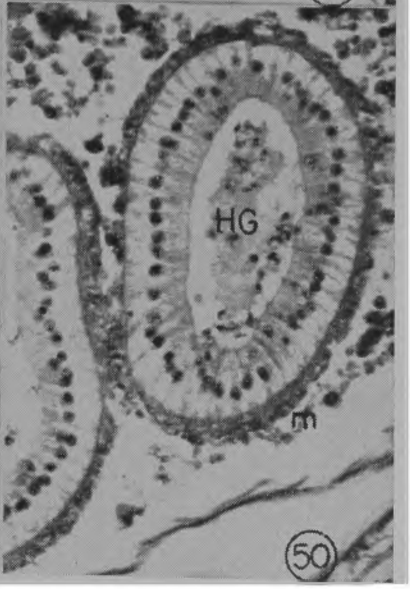
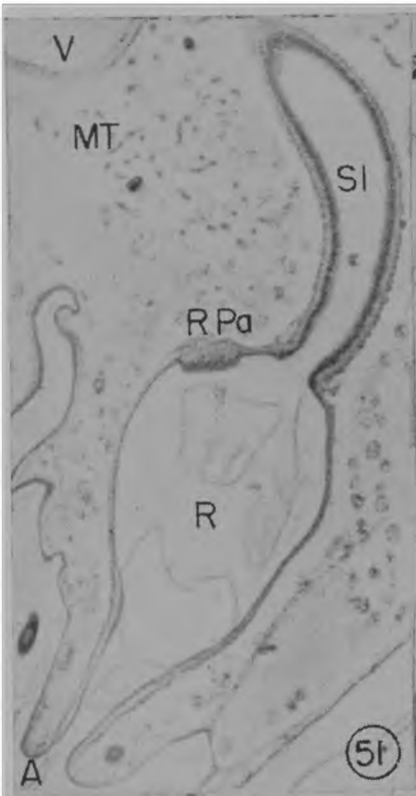
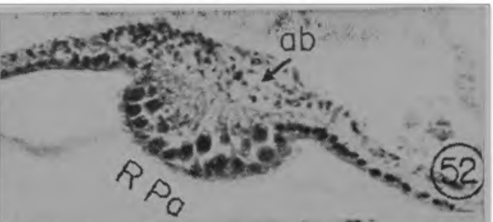
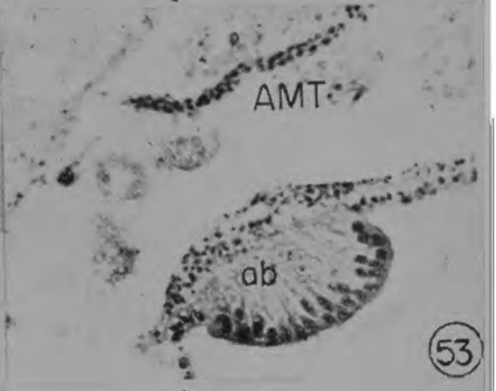
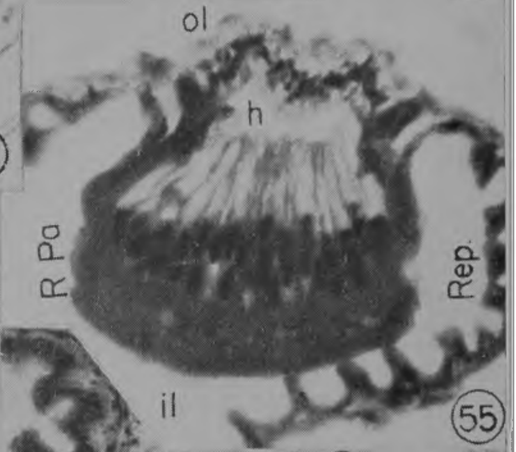


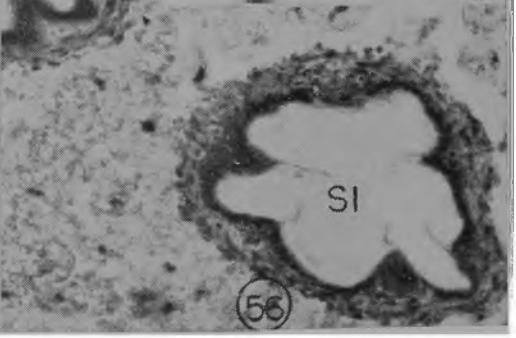
Plate 11
Figs. 51-56

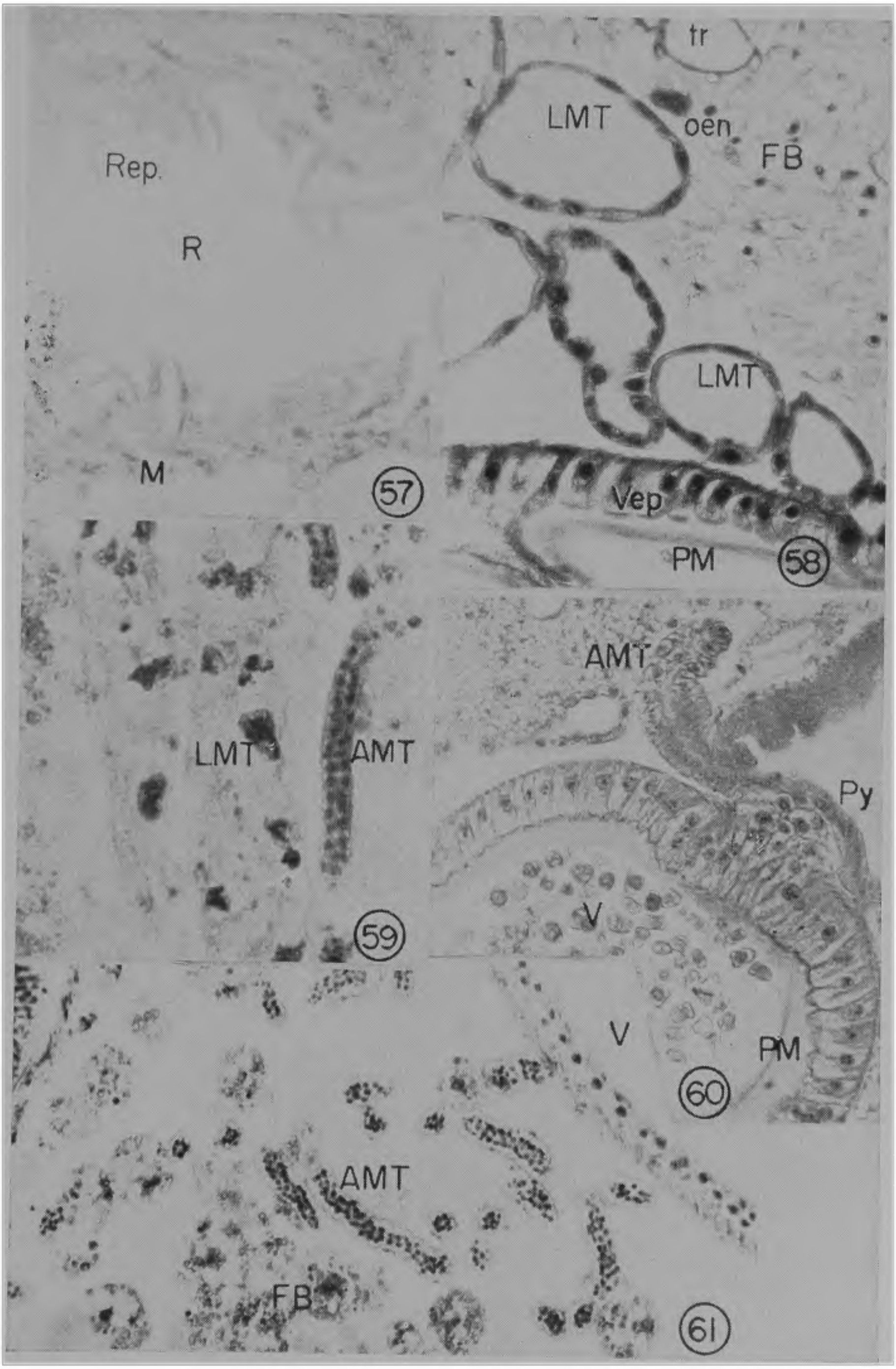








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