STUDIES ON THE MALPIGHIAN TUBULES OF INSECTS

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By
U. V. K. MOHAMED

Department of Zoology
Aligarh Muslim University,
Aligarh.

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ABSTRACT

The malpighian tubules have been the subject of interest of several workers for a long time. Cellular organization of malpighian tubules and crypto-nephric complex have been studied from several view point. The most interesting observations from purely structural point are those of Bodenheimer (1924), Marcus (1930), Green (1931), Wigglesworth (1931), Lison (1937 a,b), and Bahadur (1961, 1964) who worked with light microscope and those of Bradfield (1953), Beams et al., (1955), Meyer (1957), and Berkaloff (1958, 1959) who worked with electron microscope. The available informations on the relationship between the histological differentiation and physiological activities except for a few studies (Ramsay, 1953b, 1955a, b; Srivastava, 1961; Wigglesworth and Salpeter, 1962; Bahadur, 1964) are inconclusive. In the present study efforts have been made to provide detailed informations on the structure of the malpighian tubules of Conocephalus indicus R. (Orthoptera), Spathosternum praciniferum W. (Orthoptera), Laccotrephes maculatus F. (Hemiptera), Mylabris pustulata T. (Coleoptera), Coccinella septumpunctata L. (Coleoptera), Callograma festiva D. (Lepidoptera), Dacus cucurbitae C. (Diptera) and Vespa bicolor F. (Hymenoptera).

The histochemical studies on the tubules are confined to a few enzymes (Mazzi and Baccetti, 1957a; Arvy, 1963) and glycogen (Gabe, 1962; Couranton, 1968b). Recently the secretion of mucopolysaccharide by the malpighian tubules has shown to be associated with spittle production (Marshall, 1964b). Very limited histochemical observations are available which are but too little to fill the vast gap in the knowledge in this field. Further, no work yet has been done on different elements of malpighian tubules though there are many histochemical studies on vertebrate kidneys (Wachstein, 1955; Sassa et al., 1958; Helmy and Hack, 1967). Therefore, histochemical studies have been undertaken on a limited scale to know the nature of tissue constituents of malpighian tubules in relation to feeding in two insects viz., Mylabris pustulata (Coleoptera) and Lacctrophes maculatus (Hemiptera) of entirely different environments and feeding habits.

There is a fair number of determinations of total nitrogen, protein and phospholipids (Slowtzoff, 1909; Jarvis, 1923; Bieber et al., 1961; Fast and Brown, 1962; Crone and Bridges, 1963; Fast, 1964). However, little work has been done on the nitrogenous end products as an aid to the study of metabolism (Brown, 1936; Powning, 1953; Nation and Patton, 1961) but hardly any paper dealing with the chemistry of excreta makes an attempt to correlate them with the normal feeding habits and chemical composition of malpighian tubules.

Similarly, determinations of individual organ protein are meagre except for the distribution of protein in the blood during development (Shigmatsu, 1960; Loughton, 1965) or for the distribution of protein in the blood during the starvation (Beadle and Shaw, 1950; Orr, 1964a,b). So it was desirable to have a study on the protein concentration of malpighian tubules to know its role in tubule physiology.

Since the phospholipids occur primarily as components of biological membranes their function and metabolism may be better understood, if their concentration under various physiological conditions are known. Malpighian tubules being an organ where biological membranes play an active role in transport (Ramsay, 1953a), secretion and absorption (Wigglesworth, 1931; Berkaloff, 1960; Srivastava, 1961; Bahadur, 1964), it is of interest to examine the total phospholipid concentration under normal as well as, experimental conditions so as to correlate its functional significance.

In S. praciniferum, 12 tubules and in C. indicus 9-12 tubules come out from each ampulla. In L. maculatus, the two tubules of each side while traversing through the gut wall fuse together to form a common duct which in turn communicates with the gut lumen. In M. pustulata and C. septumpunctata and larval C. festiva there are 6 malpighian tubules. In D. cucurbitae there are only four tubules. In V. bicolor the number is found to vary from 156-182 in male and 211-252 in female. It is

proposed that the small number of tubules in Hemiptera, Coleoptera and Diptera is not primitive condition but is due to failure of secondaries to develop or reduction in the number of secondaries. In L. maculatus the four tubules at their distal ends fuse to form a common chamber. Many morphological variations are found in the same tubule at different levels. The present writer observed that these morphological variations donot necessarily conform with the histological divisions as examplified by C. septumpunctata. Similarly, in S. praciniferum, C. indicus and V. bicolor externally the tubules donot show any subdivisions but histological distinctions are possible. The present observations make it clear that the number of cells encircling the tubule differs greatly. Though the abrupt change in the type of cells from one region to the other has been noted in the present study, it is also observed that the tubules show gradation of characters within single region as found in the first region of the tubule of L. maculatus and in the second region of C. septumpunctata. It may be concluded that the tubule cells show tendency to become modified in sequential order to cope with the varying needs. Different types of cells are also found in the same region of the tubules of C. indicus.

Since the cell shows difference at various levels, it has been divided for convenience into a basal zone, central zone and border zone. The basal zone is characterised by the presence of many infoldings of the cell membrane. The central zone

cytoplasm differs in nature and granulation not only from insect to insect but also in different regions of the tubules of the same insect. In the central zone it is observed that the physiological needs influence the nature of the cytoplasm and its contents. The cytoplasm of this zone generally exhibits various types of granules.

Striated border zone is present in all the regions of the tubules of insects presently studied except in the first region of the tubule of L. maculatus, in the third region of the tubule of M. pustulata and in the third region of the right tubule of D. cucurbitae. The nuclei occupy a place in the central zone of cytoplasm. In size location and nature of the distribution of chromatin materials they differ not only from insect to insect but also in different regions of the same tubule. C. festiva larva exhibits a peculiar type of nucleus which measures 26-29 μ in length. Binucleated condition is also met with in certain cases.

Besides the peritoneal layer, in Orthopteran insects a muscular strand is found to run spirally around the tubule. The muscle fibers of the tubule are found to arise from the muscle layer of the gut epithelium.

The lumen of the tubule, ampulla or common duct is invariably found to communicate with the lumen of the hindgut either posterior to the root of the ventricular valve or

anterior to the protodaeal valve. It is therefore, suggested that the malpighian tubules are ectodermal in origin.

The distal portions of the tubules in C. septumpunctata, M. pustulata and larval C. festiva in combination with the hindgut form a crypto-nephric complex, which play a vital role in reabsorption as well as, exchange of materials. The pronephric sheath forming an envelope around a portion of the hindgut encloses the nephric tubules. There are hyaline structures in the form of modified cells called leptophragma in the nephric tubule of Coleoptera where the tubules become attached to the pronephric epithelium of the crypto-nephric complex. In C. septumpunctata leptophragma push the outer membrane from below thereby making the surface of the membrane uneven. In the case of lepidopterous larva the nephric tubule after entering into the crypto-nephric complex runs in compartments.

It is postulated that the rectal epithelium of the crypto-nephric complex is highly selective as demonstrated through experiments. Similarly, it is suggested that active transport of ions is effective through leptophragma.

Through histochemical studies it has been shown that PAS positive substances of the outer coverings of malpighian tubule of both M. pustulata and L. maculatus contain a carbohydrate which is not glycogen or acid mucopolysaccharide but

is actually a muco-complex in combination with protein. PAS positive granules found in the free tubules of M. pustulata and the tubules of L. maculatus are found to be glycogen. It is observed that in M. pustulata, muco-complex appears to play a significant role in the tubule physiology. The possible role of muco-complex as reported by earlier workers has been critically examined. Two or three Feulgen positive stained spots are found in the free tubules and crypto-nephric complex of M. pustulata whereas, in the malpighian tubules of L. maculatus it is found in the form of many granules. Though DNA is not reported to play any significant role in the tubule physiology a decrease in the intensity of staining nature of DNA after starvation has been observed in the present study.

There is a higher RNA content both in the tubules of M. pustulata and L. maculatus. It is interesting to note sudden enlargement of certain cells both in the free tubule and pronephric epithelium with RNA stained materials. It is suggested that the increased RNA activity in these cells may be due to the enhanced synthesis of certain enzymes. Since RNA is also found in the cytoplasm of the distal region immediately after feeding and in the proximal region of the tubule during starvation it is therefore, suggested that RNA plays an active role in the cellular physiology.

Biochemical studies in M. pustulata and L. maculatus have shown that total tissue nitrogen varies according to the

feeding condition. The increase in the concentration of total nitrogen in the blood and tissue after feeding and decrease in the total nitrogen during the period of starvation is more or less consistent. The concentration of the tissue nitrogen is lower than that in the blood except when M. pustulata is starved. During extreme starved condition no nitrogen is detected in the blood with the method used in the present study. The higher amount of nitrogen in the tissue than in the blood of M. pustulata and lower amount of nitrogen in the tissue than blood of L. maculatus during starvation may be due to the different environmental conditions in which they live. It is also suggested that the low concentration of nitrogen in the blood of M. pustulata may be due to the utilization of stored nitrogen during starvation. It is also found that nitrogen in the excreta of L. maculatus is higher than that of M. pustulata. The possible reason for this difference is discussed.

During starvation period there is a great reduction in the concentration of protein both in the tubules of M. pustulata and L. maculatus. Protein rise in the tubule during fed condition may be due to the increased enzyme synthesis in the tubules. It is also suggested, that there are chances for the protein to be sequestered from the haemolymph to the tissue in fed condition. Since proteins are used extensively during starvation the concentration of protein in the blood and tissue becomes low.

Both in the M. pustulata and L. maculatus there is fall in the concentration of phospholipid in the tissue during starvation. The loss of phospholipid in the tissue during the period of starvation is not so significant in contrast to nitrogen and protein. An increase in the concentration of phospholipid has been observed both in the blood and tissue immediately after feeding the previously starved insects. It shows that the phospholipid also play an important role in the tubule physiology. Similarly, it is also assumed that the higher amount of mitochondria in the tubule is probably responsible for the higher concentration of phospholipid than nitrogen in the malpighian tubule.

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HISTORICAL REVIEW

Malpighi (1969) in his work on silk worm refers to the malpighian tubules as 'vasa varicosa'. Plateau (1874) was the first person to suggest an excretory function to malpighian tubules followed by Schindler (1878) and Bordas (1911). The number of malpighian tubules occurring in various insects has been described by Wheeler (1893) and Venezeani (1904). Apterygotes in general are recorded by Tillyard (1930) as having six malpighian tubules though four are found in some Lepismatidae, sixteen in Campodea and twenty in Machilis. Marshall's (1927) study is restricted to Ephemerida in which the number varies from 40-100 whereas, Thomsen's (1947) observations are confined to the Chalcid larva where he found 2 to 3 tubules. Efforts have also been made by several workers to ascertain the primitive number of malpighian tubules. Wheeler (1893) concluded that the primitive number of malpighian tubules in insects was probably six. According to Cholodkowsky (1887) the primary tubules in Tineola and Galleria larvae are replaced by the secondaries at the time of metamorphosis. Henson (1944) suggests the primitive arrangement in insects as six primary tubules with numerous secondaries.

The malpighian tubules have long since been recognised as mesenteron appendages in Crustacea and Arachnida. It is only in Insects where an ectodermal origin (from proctodaeum) has been claimed for them. This is rather curious in view of the fact that they are attached to the mesenteron and not to the proctodaeum (Henson, 1932). Wheeler (1889) on the other hand states that tubules arise from the proctodaeum and are thus ectodermal in nature. Carriere (1890) and Nelson (1915) have reported that in Chalicodoma and Apis respectively, these tubules actually arise from the ectodermal mass before the formation of proctodaeum. Later Drummond (1936) in Lepidoptera, Thomas (1936) in Orthoptera, Paterson (1936) in Coleoptera, Sander (1956) in Homoptera, Farooqi (1963) in Hymenoptera have attributed ectodermal origin to the tubules. Henson (1932, 1944, 1946) however, has calimed endodermal origin for the malpighian tubules, thus keeping the insect in line with Crustacea and Arachnida. The endodermal nature of the tubule has also been asserted by Trapmann (1923), Tirelli (1929), Weil (1935), Alam (1953), Dhillon (1966) and Zakaur-Rab (1971).

Malpighian organs of insects are typically long, much convoluted tubules frequently branched or disposed in clusters. But the structure and arrangement vary considerably.

In Protura according to Berlese (1910) they are represented by six small oval masses of cells projecting from the anterior end of the proctodaeum; each organ consisting of two large outer cells supported on a peduncle of slender cells extended from the intestinal epithelium. In Japigidae the organs are small but tubular. Silvestri (1905) and Tillyard (1930) have described these as six very short diverticulae arising from the anterior end of the proctodaeum. Malpighian tubules are generally devoid of branching but at times these may be arborescent as in Galleria mellonella or they may give off short closely packed diverticulae as in Melolontha (Ehrenberg, 1914). When tubules are numerous these are arranged in bunches and each bunch discharging by a separate duct or 'ureter' into the alimentary canal. In Gryllidae all the ducts converge into a common 'ureter' of considerable length (Imms, 1957).

Generally malpighian tubules lie free in the body cavity but in some insects their posterior ends are anchored superficially to the wall of the rectum. This condition occurs in the larvae and adults of Coleoptera (Marcus, 1930; Poll, 1932; Stammer, 1934; Lison, 1937a,b; Saini, 1954; 1964; Ramsay, 1964). The posterior most region of the tubule is separated from the haemolymph by perinephric membrane to

which the tubules are attached through leptophragma. Between the gut wall and tubule there is a layer of connective tissue which occupies the space between the gut wall and perinephric membrane (Saini, 1964). Physiology of the crypto-nephridial system has been worked out by Pradhan (1942), Saini (1964) and Ramsay (1964). These workers agree that 'crypto-nephric system' enables the insect to conserve water by withdrawing it from faeces but there are controversies regarding the physiological processes.

According to Wigglesworth (1965) the wall of the tubule may be made up of a single or a set of cells. The cells are anchored to the basement membrane in such a manner that vertical or radial striations are formed in the basal region of cytoplasm (Bordas, 1911; 1916). The cytoplasm of the cell has varied appearance. It may be diffusely tinged with green or yellow; more often it is colourless. Sometimes almost clear but usually more or less filled with refractile or pigmented concretions or droplets and occasionally with needle like crystals (Wigglesworth, 1965).

Beams et al (1955) have worked out the structural details of the malpighian tubules of grasshopper, Melanoplus

differentialis differentialis. The apical border of the cell is composed of many parallel protoplasmic processes 3-4 u long, lying perpendicular to the tubule axis. These processes get distended due to the migration of mitochondria into this region. It is conjectured by Beams et al (1955) that mitochondria flow into these processes and eventually are thrown into the lumen of the tubule by pinching of the bulbous end. Bradfield (1953) in a preliminary study of the malpighian tubule of Galleria mellonella also has observed accumulation of mitochondria in the same manner but does not assign any reason to it. Meyer (1957) has done a comparative study of Carausius morosus, Apis mellifera, Bombus sp. and Blatta orientalis. In C. morosus he found the secretions to consist of numerous intra-cellular granules. These are compacted in the lumen to form elliptical borders. The mitochondria are appressed to the distal end of the filaments but evidence of actual discharge into the lumen could not be found. In Apis intra cellular inclusions are observed in the cytoplasm. The brush border is well defined but mitochondria are not seen in them. In Bombus also there are many inclusions of characteristic nature and these inclusions along with mitochondria seem errupting through the ruptured cell borders into the lumen of the

tubule. In Blatta orientalis the relationships are similar to those in Bombus except that intracellular granules in Carausius morosus. Meyer concludes that in none of these cells is there any golgi apparatus described for vertebrates or insects. But Mazzi and Baccetti (1963) have clearly shown golgi bodies in the tubular cells of Dacus oleae.

According to Berkaloff (1958) fine structure in these cells of malpighian tubules of Acheta domestica have some thing common with the common proximal loop of the renal tubule of vertebrates. Two types of granules; one yellow in colour and the other composed of concentric 'shells' of greatly differing electron opacity are recorded in the cells of the tubules. They arise in a complex of double membrane in the periphery of cells. These 'shells' give a positive result with murexide test. They migrate to the centre of the cytoplasm by a rupture in enveloping membrane and are ultimately eliminated into the lumen of the tubule.

Using Regaud's technique, Monterio (1957) has studied the mitochondria of Stegobium panceum. The number and position of mitochondria observed by him agree with those of Beams et al (1955). Many mitochondria have been observed breaking down to form amorphous deposits as seen in

malpighian tubules of Macrosteles (Smith and Littau, 1960), grasshoppers (Tsubo and Brandt, 1962) Drosophila (Wessing, 1962) and Rhodnius (Wigglesworth and Salpeter, 1962) which are comparable with lysosomes, described in the tissue of Vertebrates. Other type of structures of similar nature which are met with may be intermediate stages between the abnormal mitochondria, laminated spheres of calcium phosphates and carbonates with a concentric lamination.

The histochemical studies of malpighian tubules are confined to a few enzymes only. Mazzi and Baccetti (1957b) have discussed the comparative histochemistry of the tubes of Blaps gibba, Mantis religiosa, Acrida bicolor and Apis with respect to enzyme activity and its localisation. Their results show marked difference in enzyme distribution. A thorough appraisal of literature shows that both histochemical and biochemical aspects need further investigation.

MORPHOLOGY OF THE MALPIGHIAN TUBULES

INTRODUCTION

The malpighian tubules have been the subject of interest of several workers for a long time but very little attention has been given to the study of the structure of vital system. Similarly, little has been done to correlate the physiological relationship with the histological differentiation of the malpighian tubule.

Many workers have described malpighian tubule in view of their disposition with respect to the gut (Sedlaczek, 1902; Mc Dunnough, 1909; Licent, 1912; Nelson, 1917). Similarly, the study of the crypto-nephric complex has been done coincidently with the gut (Ramdhor, 1811; Dufour, 1834; 1843; Mobusz, 1897). Only a few workers have dealt exclusively with the structure and function of malpighian tubule in detail. The works of Cholodkowsky (1887), Bordas (1894, 1898, 1911, 1916), and Wigglesworth (1931) deserve special mention as also those of Marcus (1930), Poll (1932, 1935), Conet (1934), Lison (1937a,b), Patton and Craig,(1939) on the crypto-nephric complex of Coleoptera and that of Metalnikov (1908), Bordas (1911), Ishimori (1924), Henson (1931, 1932, 1937), Wigglesworth (1932), Poll (1938) and Saini (1964) on Lepidopterous larvae.

Cellular organization of the malpighian tubules have been studied from several points of view. The most interesting observations from a purely structural point are those of Bodenheimer (1924), Marcus (1930), Green (1931), Lison (1937a,b), Bahadur (1961, 1964) who worked with light microscope and those of Beams et al (1955), Berkaloff (1958, 1959) Bradfield (1953) and Meyer (1957) who have worked with electron microscope.

Although there are quite a few morphological and histological studies on the malpighian tubules the question still remains unanswered whether the histological difference met within the tubule of different insect are of the same nature and function and whether the tubule in different insects open into the hindgut or midgut?

The available information on the problem of relationship of the histological differentiation and physiological activities except for a few studies (Wigglesworth, 1931; Ramsay, 1953b, 1955a,b, Bahadur, 1961, 1964; Wigglesworth and Salpeter, 1962; Srivastava, 1962) is inconclusive. It is therefore, worthwhile to take up the study of physiological activities of the malpighian tubules and correlating them with histological variations in different groups of insects. For this a basic knowledge of morphology and histology of

the malpighian tubules of different insects is essential. So in this work besides the anatomical peculiarities the histological details have also been worked out. In the present study efforts have been made to cover the morphology and histology of Conocephalus indicus (Orthoptera), Spathosternum praciniferum (Orthoptera), Laccotrephes maculatus (Hemiptera), Mylabris pustulata (Coleoptera), Coccinella septumpunctata (Coleoptera), Callograma festiva (Lepidoptera), Dacus cucurbitae (Diptera) and Vespa bicolor (Hymenoptera). These species have been selected to give representation to all the major orders of insects.

MATERIALS AND METHODS

Insects were collected from Aligarh Muslim University Campus and reared in the laboratory. The insects were anaesthesized in ether vapour. To facilitate the study of tubules 50 u liters of 0.01% methylene blue was injected through the sides of the second thoracic segment, into the haemocoel with the help of a microsyringe. The stain was found helpful in tracing the convoluted tubules lying in the haemocoel in association with other organs.

After 10-15 minutes the insects were dissected out under a binocular microscope, and the tubules were uncoiled as quickly as possible in the insect Ringer's solution. The latter helped in maintaining the ionic balance of the system for a sufficiently long period. The tubule along with a portion of the alimentary canal was separated out and mounted either in Ringer's solution or in Glycerine jelly for the study of fresh tissue. Fresh tissue was always used to study the connection between the alimentary canal and the tubules. Due to shrinkage after fixation, often the exact nature was found to become obscure. For fixation, the tissue after its separation from the body was transferred immediately

to the fixative. For making the whole mounts the fixative used were Bouin's fluid (alcoholic) and Carnoy's fluid. Carnoy's fluid gave good results. The tissue was fixed only for half an hour and then washed with ethanol to remove the fixative. Later these were hydrated through the usual grades of alcohol and stained with Borax carmine.

For the entire mounts of crypto-nephric complex the alimentary canal was dissected out and placed in a wax dish having Ringer's solution. The pronephric sheath was torn longitudinally from one side and raised above the intestine with a pair of forceps. The intestine was ligatured at its posterior end to separate out the crypto-nephric complex from the remaining tissue of the gut.

For histological studies many fixatives like Helly's fluid, Zenker's fluid, Bouin's fluid (alcoholic), Carnoy's fluid, and Carnoy-lebrum were used. Helly's fluid and Carnoy's fluid proved good both for cytoplasmic as well as nuclear differentiation. The tissue was fixed only for 12 hours in Helly's fluid and washed for 24 hours in distilled water to remove the fixatives. Specimens were dehydrated through the usual grades of alcohols and cleared in methyl benzoate with celloidin for 15 minutes and treated with benzene for 5 minutes, infiltrated with paraffin and

finally embedded in pure paraffin. Sections were cut by rocking microtome and were stained with Delafield's Haematoxylin and counter stained with eosin.

RESULTS

THE MALPIGHIAN TUBULES OF CONOCEPHALUS INDICUS REDT (ORTHOPTERA, TETTIGONIIDAE)

Anatomy:

The excretory system of Conocephalus indicus Redt consists of a number of long malpighian tubules arising from six ampullae three on either side of the gut (Plate I, Fig. 1, Mal). The ampullae (Amp) on each side arise laterally in a row from the posterior end of the midgut. The narrow proximal portion of each ampulla gets attached to the gut. From the distal region of each ampulla 9-13 tubules arise. The number of the tubules running anteriorly or posteriorly is not constant. In certain instances six tubules are found to run anteriorly and seven tubules run posteriorly whereas, in some other cases five tubules run posteriorly and six tubules run anteriorly. But it is observed that the total number of tubules which run anteriorly and posteriorly from six ampullaeis always half of the total number of malpighian tubules present in the insect.

Each tubule throughout its length is traversed by a spirally coiled muscular strand (Plate I, Fig. 2, mcl). The

tubules are longer in females measuring 12-13 mm in length in comparison to 9.5-10.3 mm in male. Tubules in both the sexes are cream-white in colour.

The tubules running in anterior direction upon reaching gastric caeca, become attached to the alimentary canal by tracheal branchings. Similarly, the tubules running towards the posterior direction reach the rectum where they are held in position by tracheae. No anatomical variations at any level of the tubule could be observed.

Histology of the tubule:

Though anatomically the tubules show no differentiation, but on the basis of histological details each tubule may be divided into four regions.

The first region (Plate I, Fig. 4 & 5) is that part of the tubule which opens into the ampulla. Peritoneal layer (Pl) is very thick and the nuclei in it are closely located. The basement membrane (BMb) is conspicuous and holds a ring of epithelial cells. Five different type of cells are observed in this region. For convenience each cell has been divided into three major zones. The basal zone (Z_1) is formed by the infoldings in the cell membrane. The central zone (Z_2) normally lodges the nucleus while the

border zone (Z_3) is constituted by striations.

The first type of cells (Plate I, Fig. 4, c_1) are of normal nature. The deeply eosinophilic basal zone is formed by the infoldings (FcMb) in the cell membrane. These infoldings are closely packed. Cytoplasm of the central zone is reticulate. Nucleus (N) found in this zone is densely packed with chromatin granules. Nuclei are more or less round and measure about 6-7 μ in diameter. The border zone has long filaments (sb) which appear to form a line around the lumen of the tubule. It is deeply stained with eosin.

The second type of cells (Plate I, Fig. 4, c_2) are the biggest ones found in this region. The cytoplasm of these cells stain very lightly with eosin but shows a positive staining reaction with haematoxylin. No infoldings of the cell membrane are found in the basal zone. Nuclei (N) are located in the middle zone and are oval shaped measuring 12-14 x 8-9 μ. Nuclei are filled with chromatin material but no trace of nucleolus could be found. In the border zone no filaments are found.

The third and fourth type of cells (Plate I, Fig. 5, c_3 & c_4) are similar in all histological details to that of second type of cells except in the border zone. In the third

type the border zone is formed of many short filaments (sb), which are held in a line towards the luminar side, while in the fourth type many vesicles arise and extend into the lumen. The outer limits of the vesicles take little stain.

The fifth type is formed by interstitial cells (Plate I, Fig. 4 & 5, Ic) with rounded nucleus in the centre. These cells are always found between type I cells. Each cell measures 3 x 3 µ. The nuclei vary from 1.5-2.0 µ in diameter. Haematoxylin stained chromatin materials are dispersed towards the periphery of the nuclei.

In width the whole tubule varies from 28-30 u while the lumen (Lum) measures 6-7 µ in diameter. The wall of the tubule is thick measuring 10-11 u.

The second region (Plate I, Fig. 6) is enclosed by thin peritoneal layer (Pl) with widely separated nuclei. The cell membrane in the basal zone possesses only a few infoldings (FcMb). These infoldings are loosely packed and take very little eosin. The cytoplasm of the central zone is dense and deeply stained with eosin. Many minute granules are found in the central zone. The oval shaped nuclei (N) are located in the central zone. Each nucleus measures 9-11 x 4 µ. The nuclei are filled with many small chromatin

granules. The border zone is formed of long filaments (sb) which are held in a line towards the luminar side.

The tubule wall is formed of 3-4 cells. The whole tubule measures 23-25 µ in thickness, but the lumen (Lum) is only 6-7 µ wide. The wall of the tubule is 8-9 u thick.

The third region (Plate I, Fig. 7) also is enclosed by a thin peritoneal layer (Pl) having widely separated nuclei. The basal zone is deeply stained with eosin. The infoldings of the cell membrane (FcMb) are closely packed. The central zone contains oval shaped nucleus (N) measuring 8-11 x 4 µ, having a big nucleolus (Nl) and many chromatin granules. The cytoplasm of the central zone is reticulate in nature. Towards the apical portion of the central zone numerous granules (GR) are scattered in the cytoplasm. The border zone is formed of long filaments stained weakly with eosin. These filaments are displaced so uniformly that they form a line at their luminar end. In this region lumen of the tubule is of the same diameter as that of the second region.

The last portion of the tubule ending blindly forms the fourth region (Plate I, Fig. 8). Here the peritoneal layer (Pl) is very thin. The infoldings of the cell membrane

are highly reduced. The cytoplasm is reticulate. The nucleus (N) is placed in the central zone. It is oval in shape and of the same size as found in the third region. Border zone is formed of short filaments (sb) taking deep stain with eosin. These filaments form a line around the lumen.

Usually only two or three cells enclose the lumen (Lum) in this region. The tubule measures 19-22 µ in diameter but the lumen is only 3-5 µ wide. The wall of the tubule is more thick and measures 8-10 u.

Histology of the basal region (ampulla) of the tubule:

Histologically the ampulla is divisible into a proximal and a distal region (Plate I, Fig. 9 and Plate II, Fig. 10). The distal portion is formed of many layers of columnar cells. The cytoplasm in this region is less dense. The elongated nuclei, measuring 12-13 x 2-3 µ, are filled with two or three chromatin granules. In the basal portion of the ampulla interspread between the columnar cells are found, aggregated together many small cells with indistinct cell boundaries (Ic). The nuclei are distinct but are aggregated together. The nuclei are oval and measure 3-4 x 2 µ. Border zone of the columnar cells show the presence of short filaments (sb) which

are free towards the luminar side. All the tubules do not emerge at the same level from the ampulla but arise from different points.

The proximal portion of the ampulla is very close to the hindgut. The epithelial cells of the wall of one side of this portion resembles very much with the cells of the hindgut. The cytoplasm is less dense and the rounded nuclei measure 5-6 μ in diameter. Only few chromatin granules are found in the nucleus. The epithelial cells of the wall of the other side resemble very much with cells of the distal portion of the ampulla.

The lumen (Lum) of the ampulla is continuous with the lumen enclosed between the hindgut epithelium and the folds of the ventricular valve (PVlv). This composite lumen measure 7-9 μ but at the proximal end it narrows to only 2-3 μ. Ampulla is surrounded by muscle layers originating from the gut wall.

THE MALPIGHIAN TUBULES OF SPATHOSTERNUM PRACINIFERUM WLK. (ORTHOPTERA, TETTIGONIIDAE)

Anatomy:

Twelve groups of long coiled malpighian tubules from the main excretory organs of Spathosternum praciniferum Wlk. (Plate II, Fig. 11 & 12, Mal). All the tubules of each group arise from a common ampulla (Amp) which extends from the posterior most part of the midgut. Usually each group is formed of twelve tubules. Occasionally variations in the number of tubules have been observed. For example in a few specimens one or two of these groups may contain 9, 10 or 11 instead of 12 tubules. Out of the 12 tubules 6 from each group travel towards the anterior side and 6 towards the posterior side.

Ampullae are arranged around the gut in a fixed order occupying dorsolateral, lateral, and ventrolateral positions. Further the ampullae are arranged in an oblique fashion. The tubule which arise anterio-laterally take anteriorly directed course while those arising posterio-laterally run towards posterior direction. The tubule running anteriorly extend upto the gastric caeca where as the posteriorly directed tubules reach the rectum.

Each tubule measures 8.32-9.5 mm in length in male and 9.2-10.4 mm in female. The tubules are surrounded by tracheal branches and a muscular strand (Plate II, Fig. 13, mcl). The latter starts from the net work of fibers arising from the muscle layer of ampulla. This muscle strand covering the entire length of the tubule takes a spiral course.

Histology of the tubule:

Histological studies shows three different regions. The first region (Plate II, Fig. 14) is the shortest of the three lying closely approximated to the ampulla. Here the peritoneal layer (Pl) is very prominent with nucleus lying close to each other. The basement membrane (BMb) bears a ring of cells. In the basal zone the infoldings of the cell membrane (FcMb) are closely packed and are deeply stained with eosin. The cytoplasm of the central zone is coarsely fine and nongranulated. Nucleus (N) is found in this zone. The nucleus is oval in shape and measures 6-8 x 5-6 μ. Many tiny vacuoles are seen in the central zone. Frequently two of the nuclei are found to lie very close to each other but in the absence of any demarcation between the cells, it is difficult to say whether the two nuclei belong to a single cell (binucleated condition) or two cells (normal uninucleated

condition). A prominent nucleolus is generally associated with the nucleus. Highly eosinophilic border zone is formed of long filaments (sb) forming a line around the lumen of the tubule. In this region the thickness of the tubule varies from 24-26 μ. The lumen (Lum) is irregular due to the extension of the lumen towards the tubular wall. The maximum width of the lumen is 12-13 μ and the minimum is 7-8 μ.

The second region (Plate II, Fig. 16) forms the major portion of the tubule. Here the peritoneal layer (Pl) is very thin with widely separated nuclei. In the basal zone the infoldings of the cell membrane (FcMb) are thickly packed, taking deep stain. Cytoplasm in the central zone is reticulate and without granulations. The central zone contains the nucleus (N) which is relatively big measuring 8-9 μ in diameter. The nuclei consist of two types of chromatin material-one is bigger and fewer in number while the other type is small but abundant. A prominent nucleolus is always present. There is a deeply stained area before the border zone with many granules (GR). The border zone consists of many short filaments (sb) which are held in a line towards the luminar side. The thickness of the tubule in this region varies from 15-27 μ. The width of the lumen (Lum) varies from 3-4 μ only. But at places the lumen gets extended towards

the tubule wall. These extensions are longer than that observed in the first region, measuring 5-7 μ in length and 2 μ in width. In certain portions of the tubule the lumen is regular. In such places the lumen measures 7-8 μ in diameter. Cell limits are not distinct.

The third region (Plate II, Fig. 15) is the posterior most region of the tubule. Here the peritoneal layer (Pl) is thin with widely separated nuclei. The basal zone takes very little stain with eosin. Here the infoldings of the cell membrane (FcMb) are loosely packed. Towards the posterior portion of the basal zone many granules (GR) get accumulated. These granules are aggregated at certain portions of the zone. In the central zone the cytoplasm is coarsely fine. Many vacuoles are seen in the cytoplasm. Nucleus (N) which lies in the central zone is more or less spherical in nature and measures 4-5 μ in diameter. Chromatin granules are thickly packed in it. Two types of chromatin granules are found in the nucleus in the manner described for the second region. A prominent nucleolus is always present. Border zone is formed of more elongated filaments (sb) which are held in a line towards the luminar side. Very little eosin is taken by these filaments. The tubule measures 18-50 μ in thickness. The lumen (Lum) which is generally uniform measures 5-6 μ in width. The cell boundaries are not distinct.

In the case of anterior tubules which extend towards the anterior region and lie in between the gastric caeca it is found that the muscle fibers (mcl) of the tubule get attached with the muscle fibers of the gut (Plate III, Fig. 17). Similarly, just before the opening of the tubule into the ampulla the muscle fibers of neighbouring tubules get connected with each other (Plate III, Fig. 19). Usually tubules in group of three are joined together by such muscular attachements.

Histology of the basal region (ampulla) of the tubule:

The wall of the ampulla (Plate III, Fig. 18 & 20, Amp) is formed of many layer of cells. But the boundaries are not distinct. The wall of the ampulla extends into the lumen as fine projections in such a way that lumen gets reduced in size. The lumen normally measures only 2-3 μ in width except between the projected areas where the lumen measures 12-13 μ. The thickness of the wall in the projected areas varies from 19-23 μ in height while the height of the wall of the ampulla in other regions varies only 5-6 μ. The cytoplasm of the cell is hyaline. Towards the apical zone the nuclei are aggregated together. The nuclear space is filled with two or three chromatin granules only. From the apical part small free filaments (sb) are found to extend

into the lumen of the ampulla. The latter is externally surrounded by muscle layers which are continuous with that of the gut.

The ampulla is directly in communication with the gut; one end of the ampulla joins the fold of the ventricular valve (PVlv) and the other attaches with the hind gut. The fold of the ventricular valve is extended into the hindgut, through which the ampulla: lumen becomes continuous with the gut lumen.

THE MALPIGHIAN TUBULES OF LACCOTREPHES MACULATUS FABR. (HEMIPTERA, NEPIDAE)

Anatomy:

The excretory system of Laccotrephes maculatus Fabr. consists of four long coiled tubules two on either sides of the gut (Plate III, Fig. 21). These tubules arise laterally from the 'pyloric region' (Py) of the gut.

Each tubule takes a definite course in the body cavity of the insect. From its point of origin each tubule runs as ascending limb towards the anterior direction upto the meta-thoracic cavity where from it turns to take a backwardly directed course. The descending limb travels back to the point of origin of the tubule where it undergoes a number of convolutions and later extends up to the rectal sac where the four malpighian tubules fuse together to form a common chamber (Cch).

Throughout the course of displacement in the haemocoel the malpighian tubules are fastened not only to the gut wall but also to the reproductive organs, fat bodies and body wall with the help of anastomosing branches of the tracheae.

The four tubules are more or less equal in length. In the case of females the tubules are longer than their counter

parts in males. Each malpighian tubule measures 33-42.5 mm and 56-58.2 mm in male and female respectively.

On the basis of anatomical differences each tubule is divided into four regions (Plate III, Fig. 21). The first region (Reg_1) is transluscent and is found immediately after its origin from the gut. The second region (Reg_2) is opaque and represents the ascending part of the tubule. The end of the ascending limb denotes the beginning of the third region (Reg_3). This region is white in colour especially when the lumen is filled with the contents. The fourth region (Reg_4) is more narrow and transluscent. The fourth region of the four tubules join to form the common chamber (Cch).

Histology of the tubules:

On the basis of the histological differences also each tubule is divided into four regions. The first region (Plate IV, Fig. 23 & 24) is that part of the tubule which joins to the gut. The proximal and distal portions of the first region differ from each other in certain minute details, otherwise the two portions broadly resemble each other. The proximal portion of the first region (Plate IV, Fig. 23) exhibits a few muscle fibers (mcl) and a covering of connective tissue (Ct). The peritoneal layer (Pl) of the proximal portion is thick. The cell limits of the peritoneal layer are not

defined. The distal portion of the first region (Plate III, Fig. 24) lacks the muscular layer as well as, the connective tissue. The peritoneal layer is very thick and exhibits the same structure as in the proximal portion.

Next to the peritoneal layer is a ring of epithelial cells supported on a delicate basement membrane (BMb). The basal zone shows only a few infoldings of the cell membrane (FcMb) which are loosely arranged and very lightly stained with eosin. Cytoplasm of the central zone is coarsely fine and contains the nucleus (N). Nuclei are always found towards the apical end of the cell. These are more or less spherical in nature and measure 9-12 μ in diameter. A few nuclear granules, besides one or two nucleoli are also observed. A border zone is highly reduced.

The cells are not demarcated by definite boundaries. But the fact that 2-3 nuclei are observed in a cross section it may be concluded that the lumen of the tubule is enclosed by only 2-3 cells. This region is more or less uniform in thickness measuring 20-23 μ. However, the lumen (Lum) of the tubule in this region shows marked variations. At places where the nuclei are lodged, the apical portion of cell is bulged out resulting in the narrowing down of lumen which measures 4-6 μ only in this region. In other regions the

lumen is 10-11 μ wide. The thickness of the wall in this region is 4-5 μ only.

The second region (Plate IV, Fig. 25) is invested by a comparatively inconspicuous peritoneal layer (Pl) with widely separated nuclei. The cells are supported on the basement membrane (BMb). The basal zone is formed of closely packed infoldings of the cell membrane (FcMb). This zone is deeply stained with eosin. The cytoplasm of the central zone is dense and nongranular. The nucleus measuring 8-10 μ in diameter is densely packed with chromatin granules. Sometimes two nuclei are found to lie very closely. The border zone is characterised by the presence of a number of free filament stained deeply with eosin.

The lumen of the tubule in this region is 29-35 u in diameter, whereas the lumen is 10-15 μ wide. The wall of the tubule is greatly thickened measuring 9-11 μ.

The third region (Plate IV, Fig. 26) forms the longest portion of the tubule branch. The peritoneal layer (Pl) is very thick with widely separated nuclei. The basal zone is very wide as the infoldings are deep and closely packed. A variety of granules are observed in this region. Cytoplasm of the central zone is reticulate in nature. The round nuclei present in this zone measure 9-11 μ in diameter. In

many cells more than one nucleus may be observed. Occasionally characteristic dumb bell shaped nucleus is found which may be responsible for the appearance of 2 nuclei in certain cells. The nuclei are filled with chromatin granules. A prominent nucleolus (Nl) is always observed in the nucleus. A deeply stained apical area is prominent. Granules (GR) are also observed in this area. Border zone of the cell is elaborate and takes deep stain with eosin. The filaments (sb) of the border adhere together so that the free end appear to form a line.

Usually 3-4 cells encircle the lumen (Lum) in this region. The width of the lumen is only 13-18 μ. The limits are faintly distinct.

Histologically the fourth region (Plate IV, Fig. 27) has many features in common with the first and second region. The peritoneal layer (Pl) is thick with closely located nuclei. The nature of the stain taken by the cytoplasm of this region is similar to that of the second region. The basal zone presents densely packed infoldings (FcMb) taking deep stain with eosin. The cytoplasm in the central zone is coarsely fine. The nucleus (N) is oval in shape and measures 7-8 x 5 μ. The chromatin granules are fewer in number. The border zone is formed of loosely packed

filaments (sb) which are free towards the luminar side. These filaments take deep stain with eosin. In the fourth region the lumen (Lum) of the tubule is 16-17 µ in diameter while the tubule is 26-29 µ wide.

The common chamber (Plate III, Fig. 21, Cch) formed by the fusion of the last regions of the four malpighian tubule resemble the fourth region in the histological details. The common chamber which is well defined measures 58-65 µ in width. The wall is also thick and measures 12-14 µ.

Histology of the basal region of the tubule:

Sections through the region where malpighian tubules communicate with the gut reveal interesting anatomical features, (Plate IV, Fig. 28a,b,c). Both the tubules of each side are seen emerging out from the gut wall independently. Within the gut wall also these are found lying separately between the muscle layers (mcl). However across the muscle layers these two tubules are found merged with each other, thereby presenting only a single common duct (CD) on either side. The common duct is further observed traversing the epithelial layer of the alimentary canal in an oblique fashion and later communicating with the lumen of the hindgut. The lumen of the common duct is very narrow measuring only 4-5 µ in diameter.

THE MALPIGHIAN TUBULES AND CRYPTO-NEPHRIC COMPLEX OF *MYLABRIS PUSTULATA* THUNB. (COLEOPTERA, MELOIDAE)

Anatomy:

The excretory system of *Mylabris pustulata* Thunb. consists of long and greatly coiled tubules whose distal ends in association with posterior intestine form a crypto-nephric complex (Plate IV, Fig. 29). The tubules superficially appear to come out from the posterior end of the midgut but actually arise from the hindgut. The tubules run along the gut; two dorsally, two ventrally and one each on the lateral sides. The tubules are attached to the alimentary canal, reproductive organs and fat bodies with the help of tracheae.

Each tubule exhibits a definite pattern of displacement in the haemocoel.. Immediately after its emergence from the gut wall it runs along the alimentary canal in anterior direction. This ascending limb (AMal) runs upto the metathorax but occasionally it may reach prothorax from where it bends back to take a posteriorly directed course. The ascending limb is almost without any convolutions. Similarly, the descending limb (DMal) upto the end of the midgut is without much convolutions, but the remaining

portion of the tubule is highly coiled. After reaching the posterior half of the intestine the tubules once again take an anteriorly directed course. After travelling a short distance three tubules (one dorsal, one ventral and one lateral) join with each other. These in combination with similarly joined tubules of the other side form a spaceous common chamber (Cch). From the posterior end of the common chamber two sets of three tubules emerge out. The latter are labelled as nephric tubules (Nt) which in combination with the posterior intestine form the crypto-nephric complex (NRc).

The ascending limb of the tubule is pale in colour and transluscent whereas, descending limb is yellowish in colour. While the ascending limb is uniform in nature, two regions can be recognised in descending limb of each tubule. Three fourths of the descending limb which is smooth form the Ist region, while the remaining one fourth of the descending limb forms the 2nd region. The latter is characterised by the presence of dialations in the wall of the tubule. The total length of the free tubule varies from 57.2-62.5 mm in male and 83.4-92.8 mm in female.

The nephric tubule in continuation with the posterior half of the intestine forms the crypto-nephric complex. The

latter includes nephric tubules (Nt), the nephric sheath (Psh), the posterior half of the intestine. Anatomically the nephric tubule forming the crypto-nephric complex is divisible into four regions. The anterior most portion of the nephric tubule entering into the crypto-nephric complex forms the first region (Plate VI, Fig. 38, Nt). It runs in a zigzag manner without giving any branches. Very rarely one or two dialations in the wall may be seen. The second region (Plate VI, Fig. 38, Nb) is marked by the presence of many dialations called nephric buds (Nb) arising from different sides of the tubule except the one facing the gut wall. A few branches also arise from the tubule with similar dialations. In the third region (Plate VI, Fig. 39) the nephric buds are in such an abundance that they become fused together and their lumen also merge with each other giving rise to a wide compound lumen. But wherever the branches are present the nephric buds retain their entities. In the fourth region (Plate VI, Fig. 40) the buds of the tubule as well as, their branches fuse with one another. Due to the enormous developments of nephric buds the whole space below the pronephric sheath is occupied by the nephric tubules.

Nephric tubule is not able to come in direct contact with haemolymph due to the presence of a pronephric sheath.

The space between the pronephric sheath and gut wall is here called pronephric chamber. The space between the gut wall and nephric tubule is termed pronephric space (Plate V, Fig. 35, Ps) and the space between the gut wall and muscle layer is termed as sub-epithelial space (sEs). The nephric tubule terminates blindly near the posterior end of the intestine. The pronephric sheath which forms the outer covering of the crypto-nephric complex is open at the anterior end. It is held in position by the tracheal net work joining it with the gut wall and nephric tubule.

Histology of the free tubule:

Histologically the whole of free tubule is divisible into three regions. The first region (Plate V, Fig. 31) lies very close to the gut wall. The peritoneal layer (Pl) which encircles the tubule in this region is very thick, Nuclei are found close to each other. The basement membrane (BMb) is very prominent and supports ring of cells. The infoldings of the cell membrane (FcMb) are closely packed in the basal zone which stains deeply with eosin. Cytoplasm of the central zone is coarsely fine and surrounds the nucleus (N). The nuclei are more or less oval in shape measuring 5-6 x 4 μ. The nucleoplasm is hyaline except for two or three haematoxylin

stained spots. The border zone is formed of many long filaments (sb) which are free at their distal ends. Lumen of the tubule in this region is enclosed by 12-13 cells. The size of the cell varies greatly so that the lumen (Lum) of the tubule becomes irregular. It measures 9-16 µ in diameter. The whole tubule measures 51-58 µ in diameter. The tubule in this region is encircled by a few muscle fibers (mcl) which are an extension of the muscle layers of the gut.

Remaining portion of the ascending limb which forms the second region (Plate V, Fig. 32) of the tubule is enclosed by a thin peritoneal layer (Pl). The nuclei of the latter are closely arranged. There is a thick basement membrane (BMb) below the peritoneal layer. The basal zone is represented by few infoldings of the cell membrane (FcMb) which are loosely packed. The cytoplasm of the central zone is dense with granules scattered in it. The nucleus is found in this zone. The spherical nucleus measures 5 u in diameter. There is an area before the border zone with dense granulation. The border zone is formed of very short filaments (sb) which are held in a line towards the luminar side. The filaments are deeply stained with eosin.

Among normal cells one or two interstitial cells (Ic) are seen in a transverse section. The normal cells measure 12-16 μ in height. These cells are cup shaped with a broad basal region measuring 8-10 μ in width and a narrow distal region which is 3 μ in width. The whole width of the tubule is 38-43 μ in diameter.

The third region (Plate V, Fig. 33) forms the last portion of the free tubule. The peritoneal layer (Pl) enclosing the tubule is very thin with widely separated nuclei. The basement membrane (BMb) is thick and holds a ring of cells. The infoldings of the cell membrane (FcMb) are closely packed in the basal zone. The central zone where the cytoplasm is reticulate lodges the nucleus (N). The cytoplasm in close proximity of the nucleus is vacuolated. The nucleus is oval in shape and measures 4-5 x 3 μ. The border zone is highly reduced. Cells are in the form of inverted cup. The whole tubule measures 43-56 μ in diameter. The lumen is more or less regular and 23-31 μ wide.

In certain physiological condition a few cells in all the regions of the tubule become greatly enlarged. These enlarged cells show a big haematoxylin stained mass which is probably the nucleus with many granules. This mass occupies most of the space in the cell.

Histology of the basal region of the tubule:

The malpighian tubules are continuous with the alimentary canal (Plate VII, Fig. 45) through an opening in the proctodaeum before the folds of the proctodaeal valve (ProcVlv). The folds of the proctodaealvalve are formed of elongated cells. The epithelial layer is protected by a thick intima (In). The cells of the tubule at the junction with the gut are 17-18 μ in length and 4 u in breadth. The cells here show the same histological structure as in the first region. The lumen (Lum) of the tubule is continuous with the composite lumen, formed by the fold of the proctodaealvalve and the midgut epithelium. Here it may be mentioned that the proctodaealvalve instead of being directed anterior-posteriorly is placed posterior-anteriorly, and in doing so it extends into the lumen of the midgut.

Histology of the crypto-nephric complex:

The common chamber (Plate V, Fig. 34) formed by the union of the tubules shows no histological difference from that of third region of the free tubule. But the lumen of the common chamber is very wide measuring 106-123 μ in length and 133-167 μ in breadth.

The nephric tubule show the same histological details through out the entire length excepting a short anterior region (Plate V, Fig. 35) where the peritoneal layer (Pl) of the tubule is distinct. The cells are supported on a basement membrane (BMb). In the basal zone, cell membrane (FcMb) infolds into a few striations. The central zone possesses dense cytoplasm where the nucleus is lodged. The nuclei present haematoxylin stained materials towards the periphery. The border zone is highly reduced.

In the remaining portion of the nephric tubule (Plate V, Fig. 36 and Plate VI, Fig. 37) peritoneal layer is undetectable. The basal zone presents short but densely packed infoldings of the cell membrane (FcMb). The central zone is formed of reticulate cytoplasm in which round nucleus measuring about 3 μ in diameter is seen. The border zone consists of many long filaments (sb) which are free at their distal ends. These filaments are stained deeply with eosin.

The epithelium as well as, the muscle layer of the intestine in the region of the crypto-nephric complex donot show any deviation from the normal structure except in the posterior most portion where the pronephric sheath is attached

to the gut wall. Here the epithelial cells are exceptionally large measuring 9-11 μ in length and 6-7 μ in breadth. The nucleus is oval and measures 4-5 μ in length and 3 μ in width. The nuclei show very few chromatin granules.

The specialised epithelial cells of the nephric tubule called leptophragma (Plate VI, Figs. 42, 43 & 44, Lph) are characteristic feature of the crypto-nephric complex. The leptophragma differ in shape and size. Usually three types of leptophragma are found. In type I (Fig. 43) cell has no stalk. The length of the cell is variable being 3-4 μ only but the width remains constant through out the entire length i.e., measuring 2 μ. The nucleus is found more or less in the middle of the cell. Type II cell (Fig. 42) is pear shaped and measures 3-4 μ in length while the breadth is variable due to the oval shape of the cell. The base of the cell is only 1 μ wide which gradually increases to 2-2.5 μ in the middle. The apical end of the cell is again narrow. An oval shaped nucleus is found in the middle of cell. Type III cell (Fig. 44) is provided with a short stalk measuring only 2 μ. However, the cells immediately attains a bulb shaped appearance. The length of the cell is 4-5 μ. The cytoplasm of the cell is hyaline in all the types. Border zone is highly reduced.

The lumen (Lum) of the nephric tubule varies greatly except at the anterior region where it is regular while in other regions the lumen is irregular due to the folds in the wall of the tubule.

The pronephric sheath of the crypto-nephric complex arise from the distal end of the posterior intestine (Plate VI, Fig. 41). It is formed of an inner pronephric epithelium (PE) followed by a pronephric membrane (PMb) and muscle layer (mcl). The cytoplasm of the pronephric epithelium is hyaline. The oval nucleus measuring 3 μ in length and 2 μ in width is found in the cells. Pronephric epithelium forms an envelope around the nephric tubules, but leptophragma of the latter penetrates through the epithelial layer and gets attached to the pronephric membrane.

A specialised zone (Plate VI, Fig. 41, sEHG) between the posterior intestine and the rectum is responsible for the formation of pronephric epithelium and the present writer regards it as a modification of the gut epithelium. Most of the cells of the gut epithelium in this region loose the cell boundaries thereby forming syncytium with scattered nuclei in it.

The outer muscles (mcl) of the pronephric wall are formed of a single layer. It originates from the circular muscles of the gut wall. In the region where the leptophragma is situated the muscle layer is absent.

THE MALPIGHIAN TUBULES AND CRYPTO-NEPHRIC COMPLEX OF *COCCINELLA SEPTUMPUNCTATA* LINN. (COLEOPTERA, COCCINELLIDAE)

Anatomy:

The excretory system of *Coccinella septumpunctata* Linn. consists of six malpighian tubules, each of which superficially appears to come out from the posterior region of the midgut but it actually arise from the hindgut (Plate VII, Fig. 46). The distal portion of the tubule enters into a complex (NRc). Immediately after its origin each tubule runs towards the anterior direction of the gut. The ascending limb of the tubule (AMal) reaches upto the metathorax and then turns back as descending limb (DMal). The ascending limb of the shorter tubule runs upto the mesothorax only and then turns back as descending limb. In both the sexes the length of the tubule is the same; the shorter ones measuring 8.7-9.3 mm and the longer tubule 14.2-16.8 mm in length.

Each free tubule is divided into three regions. The first region (Reg_1) starting from the gut is very short. This region is whitish in colour. The remaining portion of the ascending limb along with 2/3rds of descending limb forms the second region (Reg_2). This region is yellowish in appearance. The tubular wall is thick. The third region (Reg_3) is small

and constitutes the remaining one third portion of the descending limb. This region is marked by the presence of bead like structures. This region is also yellow in colour.

The tubules are flexuous and thread like structures bathed by the body fluid. Each free tubule is adhered to the gut and reproductive organs with the help of tracheal ramifications.

The crypto-nephric complex is formed of nephric tubules, pronephric sheath and the gut wall. The narrow descending region of the posterior intestine marks the place where nephric tubules enter into the complex.

On the basis of the anatomical variations the nephric tubule is divided into three regions. The first region (Plate VII, Fig. 52) is represented by the anterior most portion of the nephric tubule immediately after its enterance into the crypto-nephric complex. This part of the nephric tubule is more or less straight and exhibits a beaded appearance. In the second region (Plate VIII, Fig. 53) nephric tubule travels in a zigzag manner. Here the dialations of the wall are more prominent. The third region (Plate VIII, Fig. 54) consists of the posterior most portion where each folds of the tubule lies perpendicular to the other.

The boursouflures are prominent as small dialations projected out from the wall of the tubule. This portion of the nephric tubule is closely adhered to the pronephric wall while the other regions are only partially embeded in the pronephric wall. The space between the pronephric sheath and the gut wall, the pronephric chamber is divided into compartments (Plate VIII, Fig. 57 & 58). Between the muscle layer and gut epithelium there is a space termed sub-epithelial space (sEs). Similarly, there is a space between the muscle layer and nephric tubule here called pronephric space (Ps). At the posterior end the nephric tubule terminates blindly. Pronephric wall is free at the anterior end. It is held in position by tracheal net work.

Histology of the free tubule:

Anatomically only three segments are distinguishable in the free tubule, but histologically it is divisible into four regions. The first region (Plate VII, Fig. 48) is the proximal portion of the ascending limb arising from the gut. The tubule is ensheathed by a peritoneal layer (Pl) which is quite thick. Above this layer lies a covering of muscle layer which is derived from the gut wall. The basement membrane (BMb) holds a ring of epithelial cells. In the basal zone the infoldings of the cell membrane (FcMb) are thickly packed. The cytoplasm of the central zone is coarsely fine. Nucleus (N)

measuring 5-7 x 4-5 μ, is seen in this region. Two types of chromatin granules are distinguished. A prominent nucleous (Nl) is always present. The border zone is very prominent and picks up deep stain with eosin. The striations (sb) are free.

The epithelial cells in the region of the junction between the gut and tubule are smaller in size but this condition prevails only for a short length after which two types of cells can be distinguished i.e., along with the smaller cells large cells are also met with. The tubule in this region measures 28-31 μ in diameter.

The second region (Plate VII, Fig. 49) is long extending upto the remaining portion of the ascending limb. The peritoneal layer (Pl) is very thin and the nuclei are widely separated. The basement membrane (BMb) supports a ring of epithelial cells. The infoldings of the cell membrane (FcMb) which are prominent are loosely packed. The cytoplasm of the central zone is reticulate with many big granules scattered in it. The nucleus (N) which is found in this zone is elongated and measures 15-18 x 8-10 μ. Two types of chromatin granules as found in the first region are also met here. The nucleolus (Nl) is prominent. Border zone is formed of many filaments (sb) which are deeply stained with eosin. These short filaments hang freely into the lumen of the tubule. The cells which

enclose the lumen (Lum) in this region are very large. The diameter of the tubule in this region is 48-51 μ. The tubule lumen is 10-14 μ wide.

In the third region (Plate VII, Fig. 50) which is formed of two thirds length of the descending limb of free tubule is covered by peritoneal layer (Pl) having widely separated nuclei. The thick basement membrane (BMb) supports a ring of epithelial cells. The infoldings of the cell membrane (FcMb) are thickly packed. It is deeply stained with eosin. The central zone is characterised by the presence of reticulate cytoplasm. The nucleus (N) situated in the middle zone is oval in shape. In the cytoplasm, variously shaped eosin stained patches are observed. Border zone is formed of short filaments (sb) which are of equal length. The diameter of the tubule in this region varies from 40-43 μ.

The fourth region (Plate VII, Fig. 51) constitutes the distal most part of the descending limb. This region possesses large cells often intermingled with small cells. It provides a spaceous lumen (Lum). The nuclei of the small cells are 5-7 x 4 μ. As far as, other histological details are concerned the cells resemble in all respects to that of third region.

Histology of the basal region of the tubule:

The tubule lumen is in communication with the lumen of the hindgut (Plate IX, Fig. 62). The lumen of the tubule before it communicates with the lumen of the intestine is narrow. Cells lining the tubule lumen present similar structural details as in the first region. At the base where free tubule comes in contact with the hindgut epithelium (HGE) the muscle layer is strengthened due to the addition of muscle bands.

Histology of the crypto-nephric complex:

The division of the nephric tubule into three anatomical regions conforms with the histological differentiations. In the first region (Plate VIII, Fig. 57) peritoneal layer is indistinct. The basal zone is highly reduced. Cytoplasm of the central zone is reticulate. A few granules are found interspread in the cytoplasm. Nucleus (N) is lodged in this zone and measures 8-9 x 4 μ. The border zone is indistinct. Lumen in this region is regular. The cell limits are inconspicuous.

The gut wall in this region of the crypto-nephric complex is greatly modified. The epithelium of the gut wall is thrown into a number of folds usually six in number. The distal portion of each such fold hanging into the lumen bears much larger cells than that of the basal portion.

In the second region (Plate VIII, Fig. 58) both the peritoneal layer as well as, the basal zone are indistinct. The cytoplasm of the middle zone is reticulate and nongranular. The nucleus is oval in shape, and measures 10-12 x 4-5 µ. The border zone is formed of deeply stained long filaments (sb) which are free towards the luminar side.

Leptophragma (Plate VIII, Fig. 55, 56, Lph) are present in this region. The cells are very small. The cytoplasm is hyaline and the nucleus is either wanting or undetectable. The leptophragma gets attached to the pronephric membrane (PMb) and in certain cases it pushes the latter towards the outerside so that the wall presents a beaded appearance. The lumen of the tubule is uneven due to the folds in the epithelium.

The epithelial cells of the gut (HGE) resemble in structure as well as, in arrangement to those of the first region except that the size of the cell here is little bigger.

The third region (Plate VIII, Fig. 59) shows no difference in the histological features from the second region except that the luminar diverticulae towards the cell wall are more in number and are closely arranged. This arrangement increases the space within the lumen.

The cells of the gut epithelium (HGE) are enlarged in size. Towards the extreme posterior end of the third region the gut wall is devoid of muscle layer, therefore, the epithelium of the nephric tubule lies face to face with the gut epithelium but in no case the two epithelia are in contact with each other.

Pronephric sheath starts from the last portion of the posterior intestine (Plate IX, Fig. 60). It is formed of a pronephric epithelium (PE) an outer pronephric membrane followed by a muscle layer. The pronephric sheath enclose the nephric tubule from all sides. Frequently leptophragma penetrates the pronephric epithelium to become attached to the pronephric membrane (PMb).

The region of the posterior intestine from where the pronephric epithelium takes origin is well defined. The epithelial cells of the gut in this region (sEHG) loose their boundaries and become syncytial in nature in contrast to the well defined cell limits in other regions of the gut wall. Immediately after its origin the pronephric epithelial cells show clear cell boundaries. This nature is met with only in one third length of pronephric epithelium. In the remaining portion, cell limits are undetectable. The circular muscle fibers (mcl) of the gut wall extend over the pronephric epithelium to provide a muscular coat. However, no muscle fibers are seen in the region of the leptophragma.

THE MALPIGHIAN TUBULES AND CRYPTO-NEPHRIC COMPLEX OF THE FULLY GROWN LARVA OF CALLOGRAMA FESTIVA DONOV. (LEPIDOPTERA, NOCTUIDAE)

Anatomy:

The chief excretory organ of the larva of Callograma festiva Donov., is in the form of six malpighian tubules, three on either sides (Plate IX, Fig. 63). The three tubules on each side actually arise as one tubule. The root of the tubule lies embedded in the gut wall from which a large pyriform ampulla (Plate IX, Fig. 61, Amp) comes out and lies on the surface of the intestine. The ampulla is joined through a narrow duct to a small but spaceous as well as, elastic reservoir (Res). From the reservoir are given off two branches. The anterior one divides into two while posterior one remains undivided. The distal portion of the three tubules in association with the rectum forms crypto-nephric complex.

Immediately after its origin each tubule runs as ascending limb (AMal) towards the anterior side of the gut upto the posterior limit of the stomodaeum from where it turns backwards and runs posteriorly as descending limb (DMal). The ascending limb is transluscent. Two thirds of the length of the descending limb is dull white in colour. The remaining portion of the descending limb is characterised by the presence

of several small diverticulae coming out from the tubule wall.

The distal portion of the tubule enters separately into the crypto-nephric complex (Plate X, Fig. 71). The pronephric epithelium (PE) soon after originating from the posterior most region of the rectum separates into two layers. Both the layers forming a loose cover over the rectal wall upon reaching the anterior end of the rectum again merge with each other and along with the other components of the pronephric sheath become finally attached to the anterior margin of the rectum. Due to the provision of two epithelial layers the pronephric chamber is divided into compartments. Each nephric tubule after entering into outer chamber runs upto the posterior limit of the rectum. The nephric tubule in this region is narrow, having very thin wall. In the posterior region of the outer chamber the nephric tubule pierces through the second layer of epithelium to enter the inner pronephric chamber. The nephric tubule is highly convoluted and runs in anterior direction upto the middle of the rectum where each tubule ends blindly.

Histology of the free tubule:

On the basis of histological details three regions are distinguished in the free tubule. In the first region (Plate IX, Fig. 64) the peritoneal layer (Pl) is indistinct. Basement

membrane (BMb) holds a ring of cells. The infoldings of the cell membrane (FcMb) are loosely packed, and are fewer in number. The central zone cytoplasm is coarsely fine and contains elongated nucleus (N) measuring 5-7 x 2 μ. But in certain cells more elongated nuclei measuring 26-29 μ are observed. The nuclei are filled with chromatin granules. Border zone is formed of short filaments (sb) which are highly eosinophilic in nature. The filaments are free towards the luminar side.

The wall of the tubule is comparatively thin and measures only about 3-5 μ in thickness, while the lumen (Lum) of the tubule is spaceous and varies from 52-63 μ in width. The whole tubule measures 58-73 μ in diameter. The cell limits are not clear.

The second region (Plate IX, Fig. 66) shows peritoneal layer (Pl) with widely separated nuclei. The basal zone is provided with thickly packed infoldings (FcMb) which are deeply stained with eosin. The reticulate cytoplasm of the central zone contains differently shaped nuclei (N). The nuclei may be dumb-bell shaped, branched or semilunar. Because of the dumb-bell shape of the nucleus, in some sections 'two nuclei' are observed lying very close to each other which are actually portions of the same nucleus. This becomes evident when the

sections pass through the middle portion. Some cell are provided with elongated nuclei. Cytoplasm presents scattered granules around the nucleus. The latter is filled with chromatin granules. A prominent nucleolus (Nl) is also present. Border zone is formed of elongated free filaments (sb) which are deeply stained with eosin.

The whole width of the tubule in this region varies from 44-59 u. The lumen (Lum) becomes irregular due to the presence of big cells among the normal small cells. The cell limits are indistinct.

The third region (Plate IX, Fig. 65) of the tubule also is encircled by a thick peritoneal layer (Pl) with widely separated nuclei. The basal zone is highly reduced. The cytoplasm of the central zone is reticulate. Differently shaped nuclei (N) are found in this zone. The nuclei are filled with chromatin granules. The border zone is deeply stained with eosin and is formed of many long filaments (sb) which are free towards the lumen. The whole width of the lumen (Lum) varies from 38-42 µ. The cell limits are indistinct.

Histology of the basal region (common duct) of the tubule:

The basal region is divisible into reservoir and ampulla. The reservoir differs in histological details from the tubule (Plate X, Fig. 69). The peritoneal layer (Pl) of the reservoir

is indistinct. The thick basement membrane holds a ring of epithelial cells whose cell boundaries are not clear. The basal zone is highly reduced. The central zone contains dense cytoplasm. Some of the nuclei (N) present in the central zone are much elongated. The border zone shows elongated free filaments (sb).

The ampulla is divisible into a body and a root. The body of the ampulla (Plate IX, Fig. 67) has a tough muscular layer (mcl). The epithelial cells are supported on a thick basement membrane (BMb). Some of the epithelial cells are greatly enlarged. The cytoplasm is coarsely fine. The nuclei (N) of the elongated cells measure 11-13 x 5 µ whereas, in the normal cells these are 5-7 x 2-3 µ.

The root of the ampulla (Plate X, Fig. 68) is a continuation of the body occupying the space between the muscle layer and the epithelium of the gut. Histology of the root is similar to that of the body of ampulla except that the muscle layer is absent. The lumen of the root is in communication with the gut lumen (Plate X, Fig. 70). Cell limits are not distinct.

Histology of the crypto-nephric complex:

The proximal portion of the nephric tubule differs histologically from the distal portion. In the proximal

region (Plate X, Fig. 71, Ant) the peritoneal layer and the basal zone are indistinct. The thick basement membrane (BMb) holds a ring of small cells, whose limits are not defined. The cytoplasm is somewhat dense. The nucleus (N) is elongated and occupies mid position in the central zone. The nuclei measure 4-5 x 2 μ. Border zone is highly reduced. The wall of the tubule is very thin. The lumen is 3-4 μ wide.

The distal portion of the tubule (Plate X, Fig. 71, Pnt) which occupies the second compartment is formed of big cells. The peritoneal layer is absent. The basal zone is deeply stained with eosin and formed of loosely packed infoldings of the cell membrane (FcMb). The reticulate cytoplasm of the central zone contains oval shaped nuclei measuring 6-7 x 3 μ. The border zone is deeply stained with eosin, and formed of long filaments (sb) which are free at their distal ends. The lumen of the tubule is irregular.

Pronephric epithelium takes its origin from the gut epithelium (Plate X, Fig. 71). Immediately after emerging from the latter it divides into two strata the inner pronephric epithelium (iPE) and the outer pronephric epithelium (oPE). Both of them are formed of single layer of cells. The outer pronephric epithelial layer is very thin and weakly developed. The inner pronephric epithelium is formed of elongated cells.

The cell limits are not demarcated. The nuclei (N) are oval in both cases. There is a longitudinal muscle layer (mcl) surrounding the outer pronephric epithelium. The muscular layer is continuous with that of the gut wall muscles.

THE MALPIGHIAN TUBULES OF *DACUS CUCURBITAE* COQ. (DIPTERA, TRYPETIDAE)

Anatomy:

The excretory system of *Dacus cucurbitae* Coq. consists of mainly four malpighian tubules two on either side of the gut. Tubules on either side arise from a common duct which itself comes out from the junction between the midgut and hindgut (Plate X, Fig. 72). Tubules of the left side differ from that of the right in many details; however the common ducts of both the sides resemble each other in all respects. The tubules coming out from the right side of the gut here referred as right tubules (RMal) are longer than those emerging from the left side of the gut, here referred as left tubules (LMal). Right tubules vary in length from 15.6-16.3 mm in male and 18.2-19.86 mm in female.

Immediately after their separation from the common duct (CD) the right tubules run along the gut wall as ascending limb upto the posterior limit of the stomodaeum where from they take a backwardly directed course. These descending limbs are convoluted and run upto the rectum where they end blindly. The ascending limb is yellowish in colour. It gives rise in an alternate fashion, small pyriform diverticulae so that

the whole region presents a beaded appearance. The descending limb is milky white in colour. Between the ascending and descending limb a small intermediate region is also recognised which is transluscent but contains a few crystals inside the lumen.

The left tubules differ in distribution from the right tubules. The right tubules after emerging from the common-duct undertake dissimilar course in the haemocoel. One of the two tubules travels for a short distance in an anterior direction and then forms a loop around the common duct of the right side, and thereafter takes a backwardly directed course. After reaching the rectum, the tubule ends blindly. The other one travels directly towards the posterior side. It is highly convoluted and ends blindly upon reaching the rectum. Both the tubules of left side are deeply yellow in colour and are of equal length. Each tubule measures 10.6-11.3 mm in male and 12.5-14.8 mm in female.

Structure of the common duct of right and left tubules is similar. The common duct is wider than the tubules but much shorter in length. It varies from 18-24 μ in thickness and measures 4.6-4.9 mm in length. Tubules are supplied with tracheal branches through out the entire length and are also firmly adhered to the alimentary canal, fat bodies, and reproductive organs.

Histology of the tubule:

On the basis of histological details two regions are distinguishable in the left tubule and four in the right tubule. The first two regions of the right tubule are similar in histological details to the I and II regions respectively, of the left tubules.

The left tubule is divisible into a proximal and a distal region. The proximal region (Plate X, Fig. 73) is the short portion of the tubule immediately after its separation from the common duct. The tubule is invested by a thick peritoneal layer (Pl). The basement membrane (BMb) holds a ring of epithelial cells. The basal zone shows a few infoldings of cell membranes (FcMb). Cytoplasm of the central zone is coarsely fine. The nucleus (N) is big and oval in shape. It measures 9-10 x 5-6 μ. Nucleus is filled with many chromatin granules. A single big nucleolus (Nl) is always found. Many granules (GR) are found in the cytoplasm. Border zone is formed of long free filaments (sb) which take deep stain with eosin.

The distal region (Plate XI, Fig. 75) is encircled by a thin peritoneal layer (Pl). Basement membrane (BMb) holds a ring of epithelial cells. Basal zone shows a few infoldings of the cell membrane (FcMb). Cytoplasm of the central zone is

more or less spherical in shape and measures 11-13 μ in diameter. The nucleus is filled with chromatin granules. The border zone is formed of a number of filaments (sb) which form a line around the lumen of the tubule.

As the cell limits are indistinct both in the proximal as well as distal regions it is difficult to demarcate one cell from the other. The tubule measures 41-43 μ in thickness. The width of the lumen shows variation in both the cases due to the enlargement of the cells having large nuclei. The lumen in this area is 4-6 μ wide in contrast to 8-9 μ wide lumen in the area of the cells which do not have nuclei.

As already mentioned the first two regions of the right tubule are similar in histological details to the proximal and distal regions respectively of the left tubule. The intermediate region (Plate XI, Fig. 76) between the ascending and descending limbs of the right tubule show two types of cells, small cells intermingled with big cells. However, these two types of cells resemble each other as far as the contents are concerned. There is a thin peritoneal layer (Pl) with widely separated nuclei. The basal zone is formed of deeply stained numerous infoldings of the cell membrane (FcMb) which are closely packed. The cytoplasm of the central zone is less dense and contains the nucleus (N) measuring 5-7 μ and 9-11 μ

in diameter in the small and big cells respectively. Large nuclei are richly packed with chromatin granules. Border zone is not distinguishable.

The peritoneal layer (Pl) in the third region (Plate XI, Fig. 77) is very thick but the nuclei are widely separated. The basement membrane (BMb) holds a circle of epithelial cells. The cell membrane forms a few infoldings (FcMb) in the basal zone. The cytoplasm of the central zone is reticulate where big nucleus (N) of 10-11 μ in diameter is situated in it. Border zone is obsolete.

The cell limits are not clear. The diameter of the distal region depends upon the amount of the content in the lumen (Lum). When the tubule is filled the diameter becomes 32-38 μ but in case the lumen is empty or with little content the diameter is only 23-27 μ. Wherever the nucleus is lodged the area of the cell gets enlarged so that the thickness of the wall in that region is found to be 15-16 μ in contrast to 4-5 μ thick wall in the portions where nuclei are not located.

Histology of the basal region (common duct) of the tubule:

The common ducts of the right and left tubules resemble each other. The proximal region (Plate XI, Fig. 80) of the common duct is formed of only one type of cells which are smaller. The distal portion of the common duct (Plate XI,

Fig. 78) is characterised by the presence of certain big cells among the normal cells. However, except for its size, these cells resemble those found in the proximal region of the common duct.

The common duct is enclosed by a peritoneal layer (Pl) having widely separated nuclei. The basal zone is formed of thickly packed infoldings (FcMb). Cytoplasm is dense in the central zone which contains spherical nucleus (N) measuring 4-5 μ in diameter. Only few chromatin granules are found in the nucleus. The border zone is formed of short filaments (sb) which are held in a line towards the luminar side.

Through out the length of common duct few interstitial cells (Plate XI, Fig. 80, Ic) are seen among the normal cells. A nucleus of 2-3 μ in diameter is found. One or two chromatin granules are seen in the nucleus.

The lumen (Lum) of the common duct measures about 20-23 μ in diameter. The diameter of the duct varies 47-52 μ. The cell limits are distinct.

From the longitudinal sections it is clear that the malpighian tubule emerges from the wall of the hindgut (Plate XI, Fig. 79). The lumen of the common duct of the malpighian tubule is in communication with the lumen of the hindgut.

THE MALPIGHIAN TUBULES OF VESPA BICOLOR FABR. (HYMENOPTERA, VESPIDAE)

Anatomy:

Long narrow tubules constitute the major excretory organs of Vespa bicolor. These tubules appear to come out from the posterior region of the midgut but actually arise from the hindgut (Plate XII, Fig. 81). The tubules are arranged in two rows. Some of the tubules take an anteriorly directed course whereas, others run posteriorly. The tubules running in anterior direction reach upto the first abdominal segment. Similarly, the tubules running in posterior direction reach upto the rectum.

Through out the course of displacement in the haemocoel the tubules are fastened to the gut wall, reproductive organs, fat bodies and body wall by means of anastomosing tracheae.

The number of malpighian tubule varies from 156-182 in male and 211-252 in female. Each tubule measures 10.6-11 mm and 13.3-14.8 mm in male and female respectively. It is lightly yellow in colour in both the sexes.

Histology of the tubule:

Histologically malpighian tubules show variation at different levels on the basis of which each tubule is divisible

into three regions. However, the first region is found to present minute variations at different levels and therefore, it is possible to make further compartments in this region.

The first region is divided into proximal, intermediate, and distal portions. The proximal portion (Plate XII, Fig. 83) shows a number of muscle fibers (mcl) running parallel to the tubule for a little distance. It is observed that these fibers arise from the muscles of the gut wall. Peritoneal layer (Pl) is inconspicuous and only the presence of one or two nuclei at different levels prove the existance of this layer. The basement membrane (BMb) holds a ring of epithelial cells. The basal zone is highly reduced. The central zone occupies the major area and lodges the nucleus (N) measuring about 3 μ in diameter. It is filled with two chromatin granules a few bigger ones and numerous smaller ones. Rarely binulceated condition is observed. The cytoplasm is coarsely fine and nongranulated. The border zone is formed of short filaments (sb) which are held together in a circle around the lumen of the tubule.

The lumen (Lum) of the tubule is enclosed by a set of 10-11 cells. These cells show a tendency to stain with haematoxylin. The whole tubule varies 22-23 μ in thickness. Lumen measures 11-13 μ in diameter.

In the intermediate portion (Plate XII, Fig. 84) the peritoneal layer (Pl) is very prominent. The cell membrane (FcMb) infolds to form a number of striations which are closely packed. The central zone consists of reticulate cytoplasm with a number of granules. The oval nuclei (N) found in the central zone measures 6-7 x 3-4 μ. Very little chromatin granules are found in the nucleus. Border zone is similar in details to that of the proximal part.

The cell limits are prominent. Usually 10-12 cells are found to encircle the lumen (Lum). The whole tubule in this region measures 28-31 μ in diameter.

In the distal portion (Plate XII, Fig. 85) the peritoneal layer (Pl) is prominent. A few infoldings of the cell membrane (FcMb) are found in the basal zone which is stained very little with eosin. The central zone is formed of coarsely fine cytoplasm. Rarely cytoplasm shows the presence of few granules (GR). Nucleus (N) is observed in the second half of the central zone. Nuclei are found and measures 4-5 μ in diameter. Border zone is more prominent and takes deep eosin. The filaments (sb) form a circle around the lumen of the tubule.

Cell limits are not clear in this region. The whole tubule measures 27-29 μ in thickness.

The second region (Plate XII, Fig. 86) shows a thin peritoneal layer (Pl). The nuclei in it are widely separated. The infolding of the cell membrane (FcMb) are very few and are loosely arranged. It is stained lightly with eosin. The cytoplasm is reticulate in the central zone. A prominent nucleus (N) 3 μ diameter is present in it. Only a few chromatin granules are found in the nucleus. Border zone is formed of many long filaments (sb) which are free at their distal ends.

There are 7-9 cells which encircle the lumen (Lum). The lumen extends into the spaces between the two cells making it irregular as well as spacious. The diameter of the tubule in this region is 26-29 u.

The third region (Plate XII, Fig. 87) is the longest region of the tubule. Peritoneal layer (Pl) is thick. The nuclei in it are closely arranged. The infoldings of the cell membrane (FcMb) are very prominent and are thickly packed. This region is deeply stained with eosin. The central zone cytoplasm is reticulate and shows the presence of many granules (GR). Nuclei (N) found in the second half of the central zone measure 4-5 μ in diameter. Many chromatin granules are seen in the nucleus. A prominent nucleolus (Nl) is always found in the nucleus. Border zone is formed of many

short filaments (sb) which forms circle around the lumen of the tubule.

The lumen in this region is enclosed by 4-5 cells. The diameter of the tubule in this region varies from 23-25 μ.

Histology of the basal region of the tubule:

The malpighian tubules in rows of two communicate with the intestinal lumen independently (Plate XII, Fig. 88). Each tubule after traversing the muscle layer (mcl) opens into the space provided by the folds of the proctodaeal valve (ProcVlv). The epithelium of the intestine in the region where the malpighian tubules communicate with its lumen is characteristic in the sense that the cells do not resemble the normal epithelial cells of the intestine. Further, these cells are different from the midgut cells. The cells are narrow and long with distinct nuclei. This region may be regarded as transitional region (Green, 1931).

D I S C U S S I O N

Number of the malpighian tubules:

The malpighian tubules have been reported in Amphipod, Crustacea, certain Arachnids and Insects. Baldwin Spencer (1885) has given an account of their anatomy in Gammarus and Talitrus. In Lithobius, there are two malpighian tubules one on each side of the body (Henson, 1932). A summary of the number of the tubules known to occur in various insects is given by Wheeler (1893) and Venezeani (1904) and an additional information on the number in Apterygota is provided by Tillyard (1930).

The number of tubules appears to be highly variable in various insects. In Spathosternum praciniferum, each bundle consists of 12 tubules in male as well as female whereas, in Conocephalus indicus, 9-12 tubules come out from each ampulla. Blatta orientalis is often stated to have 60-70 tubules (Schindler, 1878); but according to Henson (1944) the number is about 130 in male and 186 in female. Bordas (1898) has estimated 90-120 tubules in Periplaneta americana but according to Crowdler and Shankland (1972) each of the six ampullae bears 24-32 tubules. Melanoplus differentialis differentialis

possesses 200-300 tubules (Beams et al., 1955) while in Schistcerca gregaria the number varies from 220-294 arranged in 12 groups, each group having 21-28 tubules (Savage, 1956).

The excretory system of Rhodnius (Wigglesworth, 1931), Jassidae (Saxena, 1955), leaf hoppers (Day and Briggs, 1958), aquatic bugs (Bahadur, 1961), terrestrial plant feeding bugs (Bahadur, 1964) and Dysdercus fasciatus (Berridge, 1965) consists of four malpighian tubules. The number of malpighian tubule in Laccotrephes maculatus is a matter of controversy. Bordas (1905) quoted by Hamilton believes that there are four malpighian tubules. Hamilton (1931) writes that "actually there are only two tubules which arise in the usual way behind the pyloric valve and after certain convolutions return and are attached to the alimentary canal immediately behind their point of origin. A somewhat similar condition is described for Haltica bimarginata by Woods in 1916". Similarly, Presswala and George (1936) in Sphaeroderma have described only two malpighian tubules. But Kurup (1961) shares the opinion of Bordas in recording four malpighian tubules in L. maculatus. Bahadur (1961) in his work based on morphology has shown four tubules in a few aquatic bugs including L. maculatus. Later, he supported his observations by embryonic study on the development of tubules in S. rusticum (Bahadur, 1968). But the present author's observations are

not in full accord either with Bahadur (1961) or Hamilton (1931). Like other Heteropterous insects L. maculatus has four malpighian tubules but the tubules on either side after traversing some distance within the gut wall join to form a short common duct. Thus the two tubules on each side actually communicate with the gut lumen through a single common duct. The fact that the distal ends of the tubule also become continuous with each other as the tubules loose their separate identities has perhaps lead Hamilton (1931) to conclude that the same tubule after certain convolutions return back to its place of origin to become attached to the alimentary canal. In S. rusticum, Bahadur (1968) has observed that these tubules arise as four small buds which elongate and at a later stage fuse distally to form a cross. But Bahadur (1961, 1968) has not mentioned the presence of a common duct or ampulla either in his embryonic study of S. rusticum or in his histological account of L. maculatus. In Dysdercus koeinigi Srivastava and Bahadur (1961) however, have shown that the four tubules arise as four minute buds in 9 days old embryo; the two buds on each side fuse together to form a vesicle from which two outgrowths arise as two tubules. In the light of the observations made by Srivastava and Bahadur (1961) it is suggested that in L. maculatus the tubules perhaps arise as two buds on each side which fuse with each other to

form a common duct from which two tubules emerge out on either side of the gut wall.

In Coleoptera most Pentamerous forms have four tubules and other groups have six but there are exceptions as Hydrophilus has six and Siatris four tubules (Snodgrass, 1935). In Adalia bipunctata (Conet, 1934) and Ceratomegilla fuscilabris (Landis, 1936), Tenebrio molitor, Anaspis regimbarte, Anaclyptus mysticus and Grammoptera ruficornis (Saini, 1964) there are six tubules. In Mylabris pustulata and Coccinella septumpunctata also there are six tubules. C. septumpunctata has been earlier studied by Pradhan (1942) and Saini (1964). The present author's observation as far as the number is concerned is in agreement with their findings.

In Diptera the larvae as well as adults have four malpighian tubules with the exception of Culex and Psychoda (Snodgrass, 1935) which possess only five tubules. In Lucilia cuprina (Waterhouse, 1950)and Dacus oleae (Mazzi and Baccetti, 1963) four tubules have been recorded. In Dacus cucurbitae, the present writer also found four tubules.

In Lepidoptera, with rare exceptions, the tubules are six in number in both larvae and adults (Henson, 1931; Mathur, 1966; Murad, 1969). In Tiniedae six typical tubules of the larva are reduced to one pair in adult (Snodgrass, 1935).

The number of tubules in different groups of Hymenoptera varies considerably. In adult Tenthredinidae there are 20-25 tubules; but in some ants only six have been recorded though in majority of ants 12-50 tubules generally arranged in 2, 3 or 4 groups are found (Snodgrass, 1935). In larval Hymenoptera the number generally recorded is four but Chalcid larvae are known to have two to three tubules (Thomsen, 1947). In Vespa bicolor it is found to vary from 156-182 in male and 211-252 in female.

It is difficult to ascertain the primary number of malpighian tubules in insects. Wheeler (1893) concludes that the primitive insects probably had six excretory diverticulae of the intestine corresponding to the grooves between the usual six longitudinal folds of the proctodaeal valve. An alternative hypothesis has been suggested by Henson (1944) that a primitive condition is six primary tubules with the secondaries added in nymphal stages to form six groups. In Formica (Perez, 1902) four primary tubules arise as the larval tubules which get atrophied at metamorphosis and replaced by four groups of secondaries which become adult tubules. Similarly, in Tineola and Galleria (Cholodkowsky, 1887) among Lepidoptera and Eristalis among Diptera (Vaney, 1902) the primary larval tubules are atrophied at metamorphosis and replaced by secondaries in

the same way as in Hymenoptera. In most Lepidoptera and Diptera, however, it appears that the primary tubules persist through all the stages of life cycle, but the secondaries are suppressed. Untill the work of Savage (1956) on Locust no such primitive type with six primaries and numerous secondaries has been fully described. It can be appreciated that the desert Locust provides hitherto a hypothetical form. Therefore, it seems appropriate to suggest that small number of malpighian tubules in Hemiptera, Coleoptera and Diptera is not a primitive condition but is due to failure of secondaries to develop or reduction in the number of secondaries as an adaptation to the physiological condition of insects.

Anatomy of malpighian tubule:

Many anatomical variations are exhibited by malpighian tubules. The loop formation in the malpighian tubule is not uncommon in insects. In Tipula oleracea (Bodenheimer, 1924), D. koeinigi (Srivastava and Bahadur, 1961), Nezara viridula (Bahadur, 1964), D. fasciatus (Berridge, 1965), tubules of each side fuse in pairs to form loops. In Melanotus communis (Wilder and Smith, 1938) the joining takes place at the level of peritoneum, and not epithelium. In Drosophila funebris (Eastham, 1925) two of the four tubules form a complete loop

having continuous lumen. Bugnion (1920) states that the four tubules in the Lampyrid beetles form two complete loops involving epithelium. In Lepidoptera (Ishimori, 1924) Hepialid moths are exceptional in that the six tubules unite distally in two groups of three each.

The precise significance of fusion between the distal segments of the tubules is not understood. In L. maculatus this region shows close resemblance to the anterior region of the tubule which is supposed to play an important role in reabsorption of fluid as it is in Rhodnius (Wigglesworth, 1931). This portion always lies superficially on the rectum. Such an arrangement is perhaps the beginning of the modification in the excretory organs through which some Holometabolan insects like Haltica (Woods, 1916), Galerucella (Heymons and Luhman, 1933) Tenebrio (Poll, 1935; Ramsay, 1964; Saini, 1964) and others have attained the more complex forms. Many intermediate stages are also found, for example the tubules in Homoptera while crossing each other join together and lumen of the two become continuous at this point (Licent, 1912). In Cicada (Hickernell, 1920; Gouranton, 1968a) the crypto-nephric complex is of primitive type. Some what different arrangement exists in some Homoptera where a portion of the tubule remains in close contact with the gut wall through the filter chamber, for example in Cercopidae (Licent, 1912). In Tibicen septendecim

(Hickernell, 1920), the malpighian tubules along with a portion of the coiled gut are ensheathed by muscle fibers. The most peculiar feature in Typhlocybinae is the absence of filter chamber and the fusion of distal ends of the malpighian tubules (Saxena, 1955).

The fact that the malpighian tubules of the right and left sides in D. cucurbitae differ in nature and size has not been mentioned by Zaka-ur-Rab (1971) however, this fact has been pointed out by Waterhouse (1950) in L. cuprina and Mazzi and Baccetti (1963) in D. oleae. Similarly, the short nature of the two tubules out of 6 in C. septumpunctata has neither been mentioned by Pradhan (1942) nor Saini (1964). In Galleria mellonella (Snodgrass, 1935) numerous irregularly branched tubules arise from each stalk. In Dixippus morosus, Ramsay (1955a) has grouped the tubules into 'superior' and 'inferior', besides a third type called the 'the appendices of the midgut'.

Externally the tubules in S. praciniferum, C. indicus and V. bicolor show no differentiation. In the tubules of L. maculatus four regions are distinguishable whereas, only three are detectable in M. pustulata, C. septumpunctata and larval C. festiva. However, in the case of right tubule of D. cucurbitae four regions are defined. Many factors like

colour, pigmentations, convolutions, size and shape of the tubule at different levels have been taken into account by various workers for defining various regions. In Rhodnius (Wigglesworth, 1931) a transluscent distal and opaque proximal regions have been observed. In leaf hoppers (Day and Briggs, 1958) the tubules are divided into wider proximal and a narrow distal region. The proximal and distal segments are distinct from each other in D. koeinigi (Srivastava and Bahadur, 1961). Malpighian tubules of mosquito larva are transparent until the fourth instar (Christophers, 1960). In Aulacophora (Shukla and Singh, 1969) a granular and non-granular division is described. In B. orientalis (Henson, 1944) and Forficula auricularia (Henson, 1946) the tubule is divisible into a short transparent upper segment and a long lower segment full of excretory materials. In Ephemerids, Saint-Hilaire (1927) has described a thin walled narrow proximal part and a thick walled wide distal part in each tubule whereas, Marshall (1927) in the larva of Heptagenia interpunctata has observed four regions in each tubule. Similar divisions have been claimed for certain aquatic bugs but only two regions for certain terrestrial and plant feeding bugs (Bahadur, 1961; 1964). The present writer is of the opinion that the external demarcations donot necessarily conform with the histological divisions as exemplified by

C. septumpunctata. Similarly, in S. praciniferum, C. indicus and in V. bicolor the tubules do not exhibit any subdivision externally but histologically divisions have been observed. Bahadur (1964) has recorded same histological features throughout the tubules of Aspongopus janus but externally he demarcates the tubules in two regions.

Histology of the malpighian tubules:

The present observation make it clear that the number of cells encircling the tubules differ considerably in different regions of the tubules of even the same insect. However, in Rhodnius (Wigglesworth, 1931), no variation in the number of cells in different segments could be observed. But in the case of B. orientalis, the 'lower' segment of the tubule is encircled by four or five cells whereas, the 'upper' segment is encircled by only three or four cells (Henson, 1944). It is in marked contrast to only two cells surrounding the lumen of the tubule in Lepidoptera (Henson, 1932; 1937). In Vespa vulgaris (Green, 1931) the tubule is formed of five or six large cells. The distal region of the tubule of Lygaeus hospes and of Leptocorisa acuta shows only 3 cells whereas, in the proximal region the number varies from 11-14 in former and 4-5 cells in latter (Bahadur, 1964). The proximal portion of the tubule of Melanotus communis (Wilder and Smith, 1938) shows 20 cells, while the distal portion shows only 6 or fewer cells.

Histological differentiations observed at different levels of the tubules in the present study have also been reported by Sirodot (1858), Kolliker (1858), Mayer (1874), Schindler (1878), Samson (1908), Licent (1912), Gorka (1914), Pantel (1914), Blunck (1924), Wigglesworth (1931), Poll (1937), Gagnepain (1956), Day and Briggs (1958), Martoja (1959), Srivastava and Bahadur (1961), Bahadur (1961; 1964); Marshall (1964a) and others in various insects. The abrupt change in the type of cells from one region to the other noted in the present study has been reported in other insects such as Ephemeridan nymph, Heptagenia (Marshall, 1927), Rhodnius (Wigglesworth, 1931), Leptocorisa, Nezara, Cletus and Dysdercus (Bahadur, 1964). In these cases there is no gradual transition but the displacement of one rarely two cells of one region to next is not an uncommon feature. This type of abrupt change is reported in the renal tubules of vertebrates (Mollendrof, 1930). But since the tubules present gradation of characters even within a single region for example the first region of L. maculatus and the second region of C. septumpunctata it may be suggested that there is a tendency in the tubule cells to become modified in sequential order to cope with the varying needs.

In C. indicus, different types of cells are observed in the same region intermingled with each other. Similarly, cells of the same region show different types of border zone.

Such an observation is perhaps not recorded elsewhere. In the case of C. septumpunctata and C. festiva the epithelial cells of the tubule differ so much in size that two types of cells are recognized within the same region. Small interstitial cells, found throughout the free tubules of M. pustulata and in the first region of the tubules of C. indicus are also described in Dromius (Schindler, 1878), in the larva of Galleria mellonella (Metalnikov, 1908), Ptychoptera (Pantel, 1914), Anopheles (Missiroli, 1927) Lygaeus hospes (Bahadur, 1964). The suggestion put forth by Wigglesworth (1965) that these cells are possibly concerned with reabsorption need further evidence. However, in the case of M. pustulata it is found that during the diuretic period certain cells are enlarged with active RNA content and later get degenerated. The enlarged cells are also found in C. indicus. The present writer suggests that these enlarged cells are derived from the interstitial cells by cytokinensis. These enlarged cells may possibly be involved in holocrine secretion which accounts for their degeneration later. It is further indicated by histochemical studies on these cells. Since the RNA content is found more in the enlarged cell, it may be suggested that some enzyme synthesis is taking place in these cells which may be involved in the metabolic processes during excretion.

The third segment of L. maculatus the entire tubule of M. pustulata, C. septumpunctata and the intermediate zone of the first region and the second region of V. bicolor exhibit the presence of descrete cells with definite boundaries while in all other cases cell limits are indistinct. In Rhodnius (Wigglesworth, 1931) the cells in both segments have clear cell boundaries. But in B. orientalis only on the basis of the presence of four to five nuclei in a section, Henson (1944) concludes that the tubule is encircled by four to five cells. The cell boundaries in Prodenia litura and Trichoplusia ni (Mathur, 1966) are more clearly marked.

In the malpighian tubule there is an elastic homogenous membrane on which the epithelial cells are supported. However, the basement membrane differs in thickness from region to region. It seems likely that this layer is a derivative of the epithelium as suggested by Wigglesworth (1956).

The epithelial cells present variable structures even in the same region of the tubule. Border zone and the granules have been taken by the earlier workers in dividing the tubules into different histological regions. Since the cells show variations at different levels it is appropriate to divide it into a basal, central and border zone. Beams et al.

(1955) label the border zone as apical zone. The cytoplasm of the distal half of the central zone in the case of the third region of the malpighian tubule of L. maculatus and C. indicus, in the second region of S. praciniferum differs from that of the proximal half.

Peculiar feature of the basal zone is the modification of the plasma membrane which is arranged in the form of infoldings. The size of the basal zone varies at different levels of the tubules. Bordas (1916) and Bugajew (1928) have described this 'vertical' or 'radial' striations as a special apparatus to anchor the cells to basement membrane. Such basal striations have been mentioned by Imms (1924) in many insect tubules, by Marshall (1927) in Ephemeridae, and by Wigglesworth (1931) in Rhodnius. No basal infoldings have been reported in Melanotus communis (Wilder and Smith, 1938). Similarly, in the present study the third region of the tubule of larval C. festiva and the fourth region of C. indicus are exceptions in that here the basal zone is highly reduced. Bahadur (1964) has labelled this region as striated border. In Aspongopus, he has also noted that it is more conspicuous in the proximal region than in the distal region. Electron microscopic studies (Beams et al, 1955; Berkaloff, 1958; Wigglesworth and Salpeter, 1962; Mazzi and Baccetti, 1963)

also point out that the plasma membrane at the base is thrown into a series of deep parallel folds which are oriented perpendicularly to the basement membrane. The difference observed in the nature of these infoldings in the present study is also found in Rhodnius (Wigglesworth and Salpeter, 1962). Since such an arrangement has been described for the vertebrate kidney (SJostrand and Rhodin, 1953) it probably explains the structural adaptations for the same physiological function in different groups of living organism. In the stained preparations (under light microscope) thickly packed infoldings stain deeply whereas, the loosely packed infoldings stain lightly. The infoldings are generally free of granules but in the case of transitional region of the tubule in D. cucurbitae, many granules are observed in this zone under certain physiological conditions. Mitochondria have been resolved in these foldings of the 'cell membrane' in electron microscopic studies (Berkaloff, 1960; Wigglesworth and Salpeter, 1962) which suggest active transport of the materials through this region.

The cytoplasm of the central zone differs in nature as well as, granulation not only from insect to insect but also in different regions of the tubule of the same insect. It is apparent from the present study that the cytoplasm may be coarsely fine, dense, reticulate, hyaline or granulated. When

sections are prepared from the tubules of L. maculatus after injecting distilled water into the haemolymph, it is found that many big vacuoles appear in the cytoplasm of both second and third regions. Similarly, the coarsely fine cytoplasm of the second region gives reticulate appearance. When the insect is starved, all the granules present in the third region disappear. In such conditions the histological distinctions between different regions become doubtful. So it is concluded that the nature of the cytoplasm mostly depends on the physiological activities taking place in the respective regions.

The cytoplasm in the central zone generally exhibits various types of granules and refractile spheres in different regions of the tubules. The present findings show that there is no specific region which can be claimed for the location of the particular type of granules, and that the latter may be dispersed in various ways. Similarly, these may be present in any region of the tubule. In L. maculatus, as described elsewhere the granules of the third region completely disappear during starvation. In M. pustulata these granules are found throughout the entire length of the tubule. Venezeani (1904) has reported that in many insects the cytoplasm of the tubule cells is not granular, except in the

'proximal' or 'lower' segments. The cytoplasm of the malpighian tubule in insects has commonly refractile concretions but these may not necessarily be the same as excretory granules in the lumen (Venezeani, 1904; Saint-Hilaire, 1927; Wigglesworth and Salpeter, 1962). Only the 'intermediate zone' (central zone) of the cytoplasm in the case of Melanoplus possesses short rods as well as, granules (Beams et al, 1955). Crystals of calcium oxalate which are birefringe, are seen in the tubule cytoplasm of Bombyx mori (Teigler and Arnott, 1972). The refractile spheres in the tubule cells are not blackened by ammonical silvernitrate in the case of mosquitoes (Wigglesworth, 1953). All these refractile granules or spheres found in different regions of the tubules are not necessarily uric acid because in aqueous fixatives like Bouin's fluid, Helly's fluid and Zenker's fluid uratic granules are soluble but when the sections are treated with these fixatives many granules are found to remain in the tubule though there is a reduction in the concentration of these granules. In Gryllus, Xylocopus, Bombus (Berkaloff, 1958, 1959) and in Rhodnius (Wigglesworth and Salpeter, 1962) there are granules composed of uric acid and calcium urate and perhaps phosphates. These workers have also noted that the dissolved uratic spheres appear in the electron micrograph as 'husks'. There are now evidences to

prove that at least some of these granules are actually uric acid. These granules in D. koeinigi (Srivastava and Bahadur, 1961) appear only in the distal region of the tubule in the second instar nymphs at 36 hours. The appearance of these granules in each nymphal instar is marked by the commencement of feeding and at the end of each instar the granules are flushed out into the lumen of the tubule. These granules when discharged into the hindgut lumen combine with certain constituents of the faecal matter to become meconium which shows that these granules are excretory products. When the sections of the tubule of leaf hoppers are resolved under electron microscope it is found to contain brochosomes which is also an excretory material (Day and Briggs, 1958). The presence of granules in the upper segment of the tubule of Rhodnius (Wigglesworth, 1931) and also in terrestrial plant feeding bugs (Bahadur, 1964) is attributed to the secretory nature of these portions of the tubule. But the present writer feels that a single factor i.e., the presence of granules can not be considered enough to justify secretory nature of the cellular cytoplasm, since the granules are found through out the entire tubule of M. pustulata and on the other hand no trace of the granules are found in the tubule of Aspongopus janus (Bahadur, 1964). It is common feature that degenerating mitochondria are broken down and

retained in the cell as amorphous deposits (Smith and Littau, 1960; Tsubo, 1961; Wessing, 1962; Wigglesworth and Salpeter, 1962). There are now sufficient evidences to believe that at least some of these granules noticed in the tubule cells are actually amorphous deposits accumulated due to the disintegration of mitochondria. Recently, Lhonore (1971) has shown that the yellow tubules excrete spheroids of calcium, potassiu, magnesium and manganese which get crystalised in the cytoplasm of the cells. So it is apparent that these granules are of heterogenous nature but most of them are the products of the metabolic activities. The fourth segment of C. septumpunctata shows some deeply eosin stained areas the nature of which is not clear.

Striated border zone is present in all the regions of the tubules of the insects presently studied except in the first region of L. maculatus, the third region of M. pustulata and in the third region of the right tubule of D. cucurbitae. It is difficult to distinguish the difference between two types of borders in different regions of fixed tissues, as reported in Rhodnius (Wigglesworth, 1931). Presence of chitinous intimal lining has been reported by Branch (1922), Snodgrass (1925) and Lewis (1926). According to Marshall (1927) and Henson (1931, 1932) chitinous intima may be present either in a portion of the tubule or throughout the entire

length specially when the tubules arise from the hind intestine. However, the present writer could not find any trace of intima in the malpighian tubules of so many insects studied.

A detailed study of the border in the tubule of Rhodnius (Wigglesworth, 1931) shows that the distal region is made up of a series of little elongated fused vesicle whereas, the proximal region is made of brush border. Wigglesworth (1931) has ascertained that the filaments are capable of showing limited contractions. Passive movements of these filaments in the lumen of the tubules of Blaps and Scaurus (Leger and Hagenmuller, 1899) are also recorded. In Anasa tristis, Breaky (1936) describes striated border all along the lumen without a comment on the nature on these striations. According to Bahadur (1964) in most of the terrestrial plant feeding bugs the structure is similar to that of Rhodnius except in Fitha ardens, Leptocorisa and Cletus where a brush border is present throughout the length of tubule and in Aspongopus where only honey comb border is present along the entire length. The view of Bahadur (1961) that the first region of the tubule in L. maculatus is a conducting and not an excretory part, (due to the absence of border) appears to be unreasonable, since the third region of M. pustulata which is found physio-

logically active is devoid of striations and histochemical studies show that the cyclical activity of the PAS positive granules, observed in other regions are also seen in the first region of the tubule of L. maculatus. Such borderless region is also described for Ephemerids (Marshall, 1927). In Ranatra filiformis and Micronecta striata the entire tubule is lined with the honey comb border whereas, in Belostoma indicum lumen of the proximal and distal region show honey comb border while the middle region has smooth border (Bahadur, 1961). The present observation on variation in the border at different regions also get support from such findings in Tettigoniidae (Davis, 1927), B. orientalis (Henson, 1944), Forficula (Henson, 1946) and Corcyra larva (Srivastava, 1962).

Electron microscopic studies confirm the presence of mitochondria in the filaments of Melanoplus (Beams et al. 1955), as well as vesicle in Dissosteria carolina (Tsubo and Brandt, 1962). The appearance of small vesicle and mitochondria in the filaments although suggest a secretory role for them but on the other hand their presence in the proximal portion of the tubule of Rhodnius is difficult to explain in the light of the belief that it is a region of fluid reabsorption (Wigglesworth and Salpeter, 1962). In Gryllus (Berkaloff, 1960)

a reduction in the haemolymph concentration leads to a vacuolation in the tubule cell cytoplasm and appearance of large vesicles in the 'microvilli'. Similarly, during diuretic period following a blood meal, vacuoles appear in the cytoplasm and aggregate around the base of striated border and vesicular swellings are found at the tips of the filaments in Rhodnius (Wigglesworth and Salpeter, 1962). As stated elsewhere, when distilled water is injected into the blood of L. maculatus such big vacuoles appear both in the second and third region of the tubule. If the nature of the cell border has anything to do with the secretory and absorptive function, it is unlikely to suggest that the difference in the nature of the border is due to physiological demarcations in the tubule. It appears from the present study that cytological differentiation exist in the tubule cells at various phases of physiological processes rather than regional differentiation in correlation with normal function.

The nuclei in all the insects, covered in the present study are lodged in the central zone of the tubule cell. In size, location, and nature of the distribution of chromatin materials these differ not only in various insects but also in different regions of the same tubule. In the first region of the tubule of L. maculatus, in the fourth region of the

tubule of C. septumpunctata, in the distal region of the right tubule of D. cucurbitae and the proximal portion of the first region of the tubule of V. bicolor the nuclei are found in the distal half of the central zone so much so that the cell is projected into the tubular lumen. But the nuclei in the third region of the tubule of C. indicus and in the second region of the tubule of V. bicolor are found in the proximal half of the central zone of the cell. In D. cucurbitae most of the space in the cytoplasm of the cells of the third region of the right tubule and whole of the left tubule is occupied by the nucleus. C. festiva presents peculiar type of nuclei which are 26-29 u long, and are variously shaped. Nuclei of varying shapes have been shown in the diagram of the distal region of the malpighian tubules of early fifth instar larva of Philosamia ricini by Srivastava and Khare (1966) without any comment in the description. Elongated nuclei are also seen in the third region of the tubule of C. septumpunctata but are comparatively smaller than that found in C. festiva. The smallest size of nuclei are observed in the interstitial cells.

The nature and distribution of the chromatin material inside the nucleus shows many variations at regional level of each tubule but in most of the cases the nuclei are filled with chromatin granules except in the interstitial cells where

the haematoxylin stained chromatin granules are dispersed only towards the periphery of the nucleus. In the second and third region of the tubule of S. praciniferum and in different regions of C. septumpunctata there are big but fewer chromatin granules besides many minute ones. The functional significance for such a difference in the nature of these granules is not understood. In the first region of the tubule of L. maculatus the nuclei are filled with dense chromatin material as in the second, third or fourth region. Only two or three big granules are found in the nuclei of different regions of M. pustulata. In Carausius morosus, Bertheau (1963) distinguishes 5 regions in the anterior part of the tubule and seven regions in the posterior part of the tubule based on the relation between nucleus and cytoplasm. The well marked difference between nuclei of 'upper' and 'lower' segments of Rhodnius observed so clearly with light microscope (Wigglesworth, 1931) have not been reported in electrone microscopic study (Wigglesworth and Salpeter, 1962). The size of the nuclei vary greatly in different regions of the tubules of certain aquatic bugs and many terrestrial plant feeding bugs (Bahadur, 1961, 1964).

The entire tubular branch, excepting the first region of L. maculatus the first region of S. praciniferum, the third region of V. bicolor exhibits binucleated condition. The presence of binucleated condition is not an uncommon feature.

In the posterior region of the tubule of Lygaeus hospes and in the proximal region of Aspongopus janus (Bahadur, 1964) and in leaf hoppers (Day and Briggs, 1958) binucleated condition is recorded. Henson (1946) has observed in Forficula that the diameter of the nuclei of the tubule at certain ecdysis becomes almost doubled possibly because of incomplete mitosis which results in some degree of polyploidy. The increase in the size of the nucleus is gradual in D. koeinigi (Srivastava and Bahadur, 1961) and in Schistocerca gregaria (Savage, 1956) but in Forficula it appears suddenly in the third instar (Henson, 1946). In Pieris brassicae (Henson, 1932) it is accompanied by lobulation and ramification as well. From these it seems evident that the binucleated condition met within the above mentioned insects and the variously shaped nuclei of C. festiva are due to incomplete mitosis which takes place during development. The dumb-bell shaped nuclei perhaps also account for binucleated appearance in certain sections as discussed in the text.

External investment of malpighian tubule:

The malpighian tubules of insects are invested by a peritoneal coat having abundant supply of tracheoles (Ehrenberg, 1914). In all insects studies in the present work, external peritoneal layer formed of minute thin squamous cells with distinct nuclei are found. In the case of M. communis, Wilder

and Smith (1938) describe this sheath as layer of thin epithelium. Bahadur (1961, 1964) does not mention this layer in his work. In Noctuid caterpillars P. litura and T. ni (Mathur, 1966) the tubule is surrounded by a peritoneal layer of very thin squamous cells with widely separated nuclei. From the present observations it is suggested that this layer perhaps plays a vital role in the physiology of the tubule since there are significant variations in its structure suiting the histological differentiation, at various levels. The presence of tracheal end cells with tracheole branches are constant features in all the insects studied. It supplies the oxygen needed for the oxidative metabolism taking place in the tubule at different physiological levels.

Except in S. praciniferum and C. indicus there is a single muscle strand which spirals the entire length of the tubule while in all other cases muscle fibers are found only in the proximal region of the tubule. These muscle fibers seem to originate from the muscle bands of the gut wall. Venezeani (1904) has recorded 'elastic fibers' in the larva of Cerambyx and 'true muscle' in the larva of Melolontha. In the 'tunic' of malpighian tubules of Dytiscus (Runguis, 1911; Korschelt, 1924) there are fine fibrillae of contractile elements. In Gryllidae there are superficial bundles of elastic fibers and deeper striated muscle fibers forming a

spiral around the entire length of each tubule (Leger and Dubosq, 1899). In honey bee (Trapmann, 1923) each malpighian tubule consists of 6 or 8 fibrillae forming a flat band arranged in wide parallels with the axis of the tubule. Only the basal stalk of the tubule in Drosophila and Calliphora (Eastham, 1925) are provided with muscle fibers. The muscles, no doubt play an important role in the peristalsis of the tubule. Recent study on the muscles of the tubule of P. americana (Crowdler and Shankland, 1972) shows that these muscle fibers are striated having sarcomere units.

The presence of a connective tissue layer which spread around the muscle layer of the gut extends to the proximal region of the tubule in L. maculatus. Bahadur (1961) has recorded this layer in the proximal region of the tubule of B. indicum. However, in no other insects connective tissue has been reported for any worthwhile discussion.

Lumen of the malpighian tubule:

Not much importance has been given by earlier workers to the lumen of the tubule through which not only excretory materials are flushed out but also many essential materials are reabsorbed. Generally it is observed that the nature of the lumen depends upon the structure of the epithelial cells lining it and also the contents present within the space. In

C. festiva the entire tubule and in D. cucurbitae the third region of the right tubule are whitish in appearance due to the particular type of excretory material present in the lumen. Size of the lumen in a single tubule varies at different levels. In many cases where the tubule wall is thick the lumen extends between the epithelial folds thereby greatly increasing the area of the lumen. Such arrangement prevails in the second region of the tubule of S. praciniferum and in the second region of the tubule of V. bicolor. Similarly, the presence of small cells in between big cells as found in the tubules of C. septumpunctata and C. festiva also increase the area of the lumen. In Melolontha, Ehrenberg (1914) has observed many diverticulae extending from the lumen. In Cercopidae (Licent, 1912) the 'distal varicose segment' of the tubule is made up of large cells with intra cellular canaliculi communicating with the lumen. In Sphingidae (Garbarskaja, 1920) many diverticulae extending from the lumen into the 'cells' are recorded.

The basal region of malpighian tubule:

The presence of an ampulla or a common duct is not a general feature as these structures are not found in V. bicolor C. septumpunctata and M. pustulata. In other insects the tubules arise from a common duct or an ampulla which is in communication with the gut lumen.

In many cases the histological structure of the ampulla as well as, the common duct differ greatly from that of the tubule. In C. indicus and S. praciniferum the structure of the cells of the ampulla are different from that of the tubules. The cells of the proximal region of the ampulla differ from that of distal region in C. indicus, S. praciniferum and in C. festiva. However, such an abrupt change is not met with in L. maculatus and D. cucurbitae where the transition is gradual from the proximal region to the distal region. Such a change is characteristic of epithelial cells of the tubule adjoining the terminal ampulla of Rhodnius (Wigglesworth, 1931) with cilia like filaments in the larva of Drosophila (Eastham, 1925).

The most interesting feature observed is the presence of a common duct traversing the gut wall of L. maculatus and in the larval C. festiva. The histology of the common duct in larval C. festiva is similar to that described for P. brassicae (Henson, 1932), P. littura (Mathur, 1966) P. ricini (Srivastava and Khare, 1966). In the case of D. oleae (Mazzi and Baccetti, 1963) an ampulla has been observed at the opening of the common duct into the lumen. No such arrangement has been found in D. cucurbitae. Histological differentiation and the formation of many projections within the lumen of the ampulla provide enough reason to believe that reabsorption may take

place in the ampulla.

Very little attention has been given to the structural relationship between the tubules and the alimentary canal. The anterior portion of the proctodaeum is often differentiated as a well defined region into which open the malpighian tubules (Snodgrass, 1935). But much variations have been observed in the course of present study as far as the communication between the tubule lumen and the gut lumen is concerned. In the case of S. praciniferum and C. indicus although the ampulla is attached externally to the posterior part of the midgut but the lumen actually communicates with the hindgut. Similarly, in L. maculatus, C. septumpunctata, M. pustulata and V. bicolor the malpighian tubule is attached externally to the wall of the posterior region of the midgut but the lumen communicates with the hindgut lumen. In the case of D. cucurbitae the common duct of the tubule although joins the gut at the junction of the mesenteron and the proctodaeum its opening is actually located in the hindgut. In the case of larva C. festiva the common duct of the tubules communicates with the proctodaeum besides being attached to the latter. In M. pustulata and V. bicolor these tubules open into the lumen of the gut above the proctodaeal valve; but in D. cucurbitae, L. maculatus and C. septempunctata the tubules open below the ventricular valve.

According to Baldwin Spencer (1885) Malpighian tubules in Gammarus and Talitrus open into the midgut. Davis (1927) in Stenopelmatus describes six 'ureters' lined with a 'kind' of epithelium, typical of midgut opening into the posterior end of the latter. In beetles, Necrophorus and Gnaptor (Gorka, 1914) the tubules open directly into the midgut anterior to the pyloric sphincter. However, the present writer could not observe in any of the insect covered in the present study, the tubules communicating with the lumen of the midgut. In Coccids, Weber (1930) is of opinion that it opens very close to the commencement of the hindgut. In Pleretes (Bordas, 1911) Hepialus (Henson, 1932) the tubules have been shown to open into the posterior most region of the midgut while in P. brassicae it has been found to open into the hindgut (Henson, 1932). The malpighian tubules of Rhodnius (Wigglesworth, 1931) open into a large 'rectal pouch'.

As mentioned earlier the tubules have been invariably found in the present study opening into the hindgut, posterior to the root of the ventricular valve or anterior to the proctodaeal valve. In the case of D. cucurbitae, M. pustulata and C. septumpunctata the opening of the tubule lies in close proximity to the midgut but a narrow strip of hindgut epithelium intervenes between the opening of the tubule and the midgut

epithelium. In other insects for example C. indicus, S. praciferum, L. maculatus, C. festiva (larva) and V. bicolor the lumen of the tubule communicates with the hindgut lumen far removed from the midgut.

From the present observation it is clear that wherever the ventricular valve is present the folds of the valve cover the opening of the tubule in such a way that the food particles are checked from entering into the lumen of tubule. In V. bicolor where the ventricular valve is absent the proctodaeal valve is directed towards the midgut. Same is the case with M. pustulata. So it appears that the control of the passage of food in these insects is done by the rectal folds. Since the food gets accumulated in this region, the opening of the tubule is liable to get blocked. However, in the absence of ventricular valve, the proctodaeal valve become anteriorly directed and take up the responsibility of guarding the opening of the tubule lumen. In the case of S. praciniferum and C. indicus these folds form a double valvular mechanism which efficiently prevents the regurgitation from the gut into the ampulla. Such a structure has also been observed in adult Forficula (Henson, 1946). In most of the insects studied the ventricular folds donot form a perfect valvular mechanism. In the case of D. cucurbitae and C. septumpunctata no folds are recognized except for the enlargement of posterior midgut epithelium which extend into the lumen of the proctodaeum.

Structurally the tubule cells resemble that of proctodaeum rather than the columnar cells of the midgut, but histological similarity hardly appears to be a sound basis for justifying common origin of organs. Similarly, the absence of an intima and the presence of striated border can not be taken as a criteria for the endodermal origin since ectodermal derivatives like prothoracic glands and accessory reproductive glands have been reported to be devoid of chitinous intima (Srivastava and Khare, 1966). The contention of the present author that the tubules are ectodermal in origin find firm support from the electron microscopic study of the rectum of Blowfly (Berridge and Gupta, 1966) which shows that the apical surface of the cell of the rectal wall consists of numerous 'microvilli'. But the presence of columnar cells in the ampulla of C. indicus indicates that the ampulla may be partially endodermal in nature. It is likely that during the embryonic development when the tubules after originating from the proctodaeum traverse the midgut in an oblique fashion, some of the midgut cells join the tubules and take part in the formation of ampulla. But it is too early to come to a conclusion since it needs further investigation.

Crypto-nephric complex:

The crypto-nephric complex of C. septumpunctata has been studied by Pradhan (1942) and Saini (1964). Amouriq (1960) has described it along with the gut. Six free tubules enter separately into the complex of C. septumpunctata as is the case with Adalia bipunctata (Saini, 1964) while in Tenebrio six free tubules fuse to form a long trunk from which arise six nephric tubules (Poll, 1935; Lison, 1937a; Ramsay, 1964; Saini, 1964) but in the case of M. pustulata six tubules fuse into two sets three on either side to form a common rectangular chamber. In certain other Coleoptera like Haltica (Woods, 1916), Agelastica, Gastroidea (Conet, 1934), Leptinotarsa (Paty, 1937) and Aulacophora (Saini, 1954) there are two tubules in the anterior set and four in the posterior set. These tubules in groups of three (one of the anterior set and two of the posterior sets) on either sides unite to form a common stem except in Timarcha (Saini, 1964) where the two anterior tubules reassociate on one side of the rectum and the remainder reassociate on the other side in groups of two each.

The distribution of the tubule in the perirectal chamber varies greatly. In the case of C. septumpunctata, the tubules run unconvoluted in the anterior region but in the second region become convoluted and the convolution become more complex in the third region. Lateral walls show only alternate diverti-

culae. The nephric tubule with many primary and secondary branches bearing bud like diverticulae as seen in M. pustulata has not been reported by earlier workers. In the Tenebrio (Saini, 1964) the nephric tubule runs in the perinephric chamber in a serpentine fashion. In the case of Hylobius and Balaninus (Marcus, 1930) there are six tubules which run around the 'perinephric chamber' but in Phyllobius (Saini, 1964) most of the tubules come to lie on one side of the gut so there is a large zone of radially disposed tubules followed by a small zone in which the tubule lie on the lateral or ventral side. According to Aslam (1961) in Araeocerus fascicatus the four tubules are radially disposed in the anterior third of the nephric chamber.

Ishimori (1924) has described the arrangement of nephric tubule in many lepidopterous larvae. The arrangement of the tubule in larval C. festiva is similar to that described by Henson (1931) in Vanessa except that instead of loops a number of convolution are formed. In Galleria (Ishimori, 1924) the tubules cover the entire length of the rectum, a condition not observed in the green feeding forms (Metalnikov, 1908; Ishimori 1924). In C. festiva the distal part of the tubule which is lodged in the inner chamber of the crypto-nephric complex extends only upto the middle of the latter, as it is in the case of Bombyx, Gastropacha and Spilosoma (Saini, 1964).

The general structure of nephric tubule in C. septumpunctata and M. pustulata is more or less the same as that described by Conet (1934), Poll (1935), Landis (1936), Lison (1937a), Pradhan (1942) and Saini (1954, 1964) in Coleoptera except for the different types of leptophragma observed in C. septumpunctata and M. pustulata and that of the larval Callograma festiva is similar to that described by Ishimori (1924), Henson (1931, 1932, 1937) and Saini (1964) for lepidopteran larvae.

Lison (1937a) was the first to describe that there are hyaline structures called leptophragma in the crypto-nephric complex of Coleoptera where the reassociated tubules come in contact with the outer membrane of pronephric epithelium. Landis (1936) regards this structure as 'nephric tubule collapsed and joined with outer membrane at some parts'. In Adalia bipunctata, Agelastica alni and Gastroidea viridula Conet (1934) has described them as small 'windows' without cells and enclosed by a very thin membrane. The explanation putforth by Saini (1964) that the cell is very small and the nucleus also being insignificant is commonly missed appears to be reasonable as observed in the sections of the tubules of C. septumpunctata. A special type of leptophragma in which the outer membrane of the pronephric epithelium is projected outside in the form of an inverted cup as described by Saini (1964) in

Dermestes lardarius, is similar to the present observation in C. septumpunctata. But Pradhan (1942) has not mentioned leptophragma in C. septumpunctata. The different types of leptophragma found in M. pustulata in the present study are also recorded in Tenebrio (Saini, 1964). In Grammoptera ruficornis, Callosobruchus analis and Callosobruchus chinensis the leptophragma is formed of a big cell (Saini, 1964). According to Poll (1935) in Tenebrio the cell is situated at one side and that the wall of the tubule is broken although covered by a thin but complete 'perinephric membrane' at this point. On the other hand Conet (1934) is of opinion that the cell is complete and not broken and that the 'perinephric membrane' does not form a cover over it. Lison (1937a) states that the cell of leptophragma lies on one side of the projection, 'boursouflures' of the median diverticula that touches the 'perisolenic' membrane. The leptophragma appears to be merely a hyaline projection of the cells of the diverticula of the enclosed tubules (Saini, 1964). Since the leptophragma penetrates through the pronephric epithelium, it appears to play some important role in the physiology of excretion. This is evident as the nucleus of leptophragma of M. pustulata under starved condition shows a very high concentration of RNA which suggest secretory role of undefined nature and therefore, the present author believes that it is a specialised cell of the median

diverticula of the nephric tubule which not only helps to attach the tubule with the nephric wall but also has an important physiological function. This view gets further support from the fact that the muscle layer is absent from the region of the leptophragma.

Marcus (1930) was perhaps the first to describe that nephric tubules are situated near the nephric membrane, namely 'tunica propria' which Pradhan has termed 'fascial envelope'. Saini (1964) uses the term 'perinephric membrane' enclosing the rectal complex and demarcates it from the pads of connective tissue. Since the membrane which covers the crypto-nephric complex when stripped off comes out as one piece including the tissue lying below it, Ramsay (1964) believes that perinephric membrane is a complex structure and is made up of laminae, the lower lamina being folded inwards to produce the so called connective tissue. The present writer is also of the opinion that pronephric membrane, pronephric epithelium and muscle layer which are inseparable from each other form a complex structure here called pronephric sheath.

From the histological sections it is evident that the pronephric epithelium is formed of many layer of cells which sometimes show regional differentiation as in C. septumpunctata. The modified cell called leptophragma penetrates through the

pronephric epithelium and comes in direct contact with the haemolymph. It is further observed that the pronephric epithelium is actually modified epithelium of the hindgut which extends towards the anterior side in the form of an envelope. At the junction the rectal epithelium looses the cell boundaries and forms a syncytium. The Dermestes, Anthrenus, Ptinus and Niptus there is a small modified zone at the posterior end of the 'descending part of the gut' where the reassociated tubules come in direct contact with the gut epithelium. Here the epithelial cells of the gut are modified into palmate myo-epithelium whose cell boundaries are not clear (Saini, 1964). All along this region in M. pustulata as well as, C. septumpunctata there is no muscle layer between the gut epithelium and nephric tubule. This feature has also been recorded in most of the insects studied by Saini (1964). Similarly, the muscle layer of the pronephric sheath is continuous with that of the gut wall. The present author's observations get firm support from studies on the embryonic development of P. ricini (Srivastava and Khare, 1966) in which the two layers of cells of the envelope (pronephric sheath) found to originate from the epithelial lining of the gut. The presence of muscle layer indicates towards the variable accommodative nature of the nephric chamber.

In the case of C. septumpunctata and M. pustulata, the gut epithelium in the region of the crypto-nephric complex is modified. While in C. septumpunctata the modification of the gut epithelium is recorded even in the anterior part of the crypto-nephric complex, in M. pustulata it is found only at the posterior end of the complex. Neither Landis (1936) in Ceratomegilla fuscilabris nor Pradhan (1942) in Coccinella septumpunctata mentioned anything about the modification of gut epithelium in their account on crypto-nephric complex. Saini (1964), however, describes 'the activated epithelium' only at the posterior end where pronephric epithelium is attached to the rectal sac. The presence of minute openings in the intimal lining of the gut providing a connection between the lumen of the hindgut and the body cavity through pronephric epithelium are recorded by Landis (1936) and Pradhan (1942). Saini (1964) has not found any indirect connection. The present author also could not find any such pores.

Ishimori (1924) has described the 'cryptonephric envelope' of lepidopterous larvae as composed of three layers, outer muscle layer, middle epithelial layer and inner membranous layer. The present observations agree with the number of layers observed by Ishimori (1924) but differ in the nature of pronephric epithelial layers. In the case of

larval Callograma the outer pronephric epithelial layer is very thin and weakly developed whereas, the inner pronephric epithelial layer is thick and formed of elongated cells. The observations of the present author are in conformity with the studies of Henson (1931, 1932, 1937) and Saini (1964).

There is much controversy regarding the function of the crypto-nephric complex (Conet, 1934; Poll, 1935; Lison, 1937a; Paty, 1937). Wigglesworth (1934) remarks that 'the precise significance of this arrangement is not known; perhaps this serve to add the absorptive powers of malpighian tubules to the rectal epithelium'. Poll (1935) believes that the broken wall of the leptophragma serves as 'glomerulus'. Pradhan (1942) believes that there are fine pores in the rectal intima through which the fluid content of the gut passes into the perirectal chamber, waste products being removed by the malpighian tubules before the fluid actually mixes with the haemolymph. Patton and Craig (1939) conclude that the reassociated tubules of Tenebrio serve only to absorb materials from haemolymph. When different dyes were injected by the present author into the rectum of M. pustulata it was found that indigo carmine appear in the nephric tubule within three minutes in the starved ones and 18-20 minutes in normally fed ones. But at the same time neutral

red which is highly diffusible and also methyl blue, congo red etc., could not pass through the rectal epithelium. It clearly shows that the rectal epithelium in the region of crypto-nephric complex is highly selective. According to Saini (1964) this 'system' replaces the rectal glands normally present in the rectum, affording a more efficient apparatus for the exchange of materials. When distilled water is injected into the lumen, it is readily removed via 'perirectal tubules' (Ramsay, 1964). The question now arises what role is played by pronephric epithelium and leptophragma. Ramsay (1964) is of opinion that in the event of water deprivation the concentration of haemolymph rises and potassium chloride from the blood is actively secreted into the tubule whereas the water is passively drawn into the tubules from the perinephric fluid. Since the leptophragma is nacked and atleast in M. pustulata during starvation period increased RNA activity is found in the nucleus, it is suggested that active transport of ions may take place through the leptophragma and pronephric epithelium. In view of these findings it seems reasonable to state that Saini's (1964) opinion that leptophragma serves only to attach the tubule to the nephric membrane is not very convincing. Similarly, in the case of larval Lepidoptera, where there is no muscle layer between the gut epithelium and the nephric

tubule active movement of fluid between the rectum and nephric chamber is probably facilitated. The experiments conducted by Patton and Craig (1939) give no indication of exchange of fluid although they have shown that there is a big turn over of body fluid which may be the outcome of this peculiar arrangement.

HISTOCHEMISTRY OF THE MALPIGHIAN TUBULES

INTRODUCTION

In insects the histochemical studies have been mainly restricted to the field of enzymes especially on digestive system. Esterases have been localised in the digestive tracts of several species of Blattidae (Day and Powning, 1949; Eisner, 1955) of Galleria mellonella (Przelecka et al., 1959) and of Dysdercus fasciatus (Khan and Ford, 1967). Mazzi and Baccetti (1957a) have found phosphatase in proximal as well as, distal portions of the malpighian tubules in Donus crinitus. However, only the proximal portion of the tubule is concerned with excretion while the distal portion produces silk. Alkaline phosphatase, acid phosphatase, lipase and succinic dehydrogenase have been identified in different regions of malpighian tubules of Acrida bicolor, Blaps gibba, Mantis religiosa and Apis mellifera (Craig, 1960). The β-Glucuronidase is reported in the tubules of several insects (Arvy, 1963).

The distribution of glycogen has been localised in the malpighian tubules of many insects (Gabe, 1962; Gouranton, 1968b). Until reviewed recently by Gabe (1962) the occurrence of muco-complex other than chitin was thought to be uncommon in insects (Day, 1949). Recently, secretion of polysaccharide by the malpighian tubules of cercopoid larvae has shown to be associated with

spittle production (Marshall, 1964b). Muco-polysaccharides also are localised in the digestive tract of Earwig, Paralabis dohrni (Joshi, 1965).

The very limited histochemical studies on the malpighian tubules make only little contribution to fill the lacunae in the knowledge in this field. Histochemical studies have shown that in different tissues of insects the physiological differences are more distinct than revealed by the simple histological studies. No work yet is available on different constituents of the malpighian tubules though there are many histochemical studies on vertebrate kidneys (Wachstein, 1955; Sassa et al., 1958, Helmy and Hack, 1967). The present histochemical studies have been undertaken to ascertain the nature of the tissue constituents of malpighian tubules in correlation with feeding in two insects namely; Laccotrephes maculatus and Mylabris pustulata inhabiting entirely different environments and having feeding habits, far removed from each other.

MATERIAL AND METHODS

Insects from culture were removed and starved individually in isolation. Mylabris pustulata were starved for three days and Laccotrephes maculatus for six days. Then the insects were allowed a full meal. At regular intervals the insects were removed from the jar and were given ether anaesthesia. The dissections were made in Ringer's solution under a binocular microscope. The tissues were transferred quickly to different fixatives for different histochemical studies.

The fixatives having been removed, the dehydration of the tissue was carried out through the usual grades of alcohol. Tissues were then cleared in methyl benzoate with celloidine for fifteen minutes, treated with benzene for five minutes, infiltrated with paraffin and embedded in pure paraffin. Sections 8-10 μ thick were cut with the help of rocking microtome.

Carbohydrate:

Periodic acid Schiff's technique (PAS) of McManus as given by Pears (1960) is employed on materials fixed in cold Bouin's fluid, Zenker's fluid, Helly's fluid, cold Carnoy's fluid and Baker's formol calcium. Bouin's fluid was found

most suitable fixative giving as a whole good results. Baker's formol calcium did not give satisfactory results as far as malpighian tubules are concerned. Parallel sections were treated at 22°C for six hours in 16 ml acetic anhydride in 24 ml of dry pyridine before staining with PAS technique.

Glycogen has been demonstrated also by best Carmine stain in tissues fixed in Cold Bouin's fluid. Control sections were incubated with saliva for two hours at 37°C before treating with the stains. Lugol's iodine is used as a test for starch. Sections were examined in glycerine jelly.

Alcian blue method of Steedman (1950) has been used to detect acid mucopolysaccharides in the freshly filtered 0.1% solution of alcian blue in 3% acetic acid; the sections were stained for 1-2 hours. After staining, neutral red was used as a counter stain. Sections were treated with 0.1% toluidine blue in 30% ethanol for 5-20 minute (Kramer and Windrum, 1956) for metachromatia. Similarly, sections were also treated with 0.5% toluidine blue for 6 hours (Pears, 1960). After staining, slides were washed in water and examined in glycerine jelly. Bouin's fluid, Zenker's fluid and cold Carnoy's fluid were used to fix the tissue.

To demonstrate separate groups of carbohydrate, sections fixed in formol alcohol, acetic alcohol, cold Carnoy's

fluid and Bouin's fluid were stained in combined dialysed iron and PAS stain (Ritter and Oleson, 1950). Tissues, fixed in Bouin's fluid and acetic alcohol gave good results. Tissues were treated only for five minutes in dialysed iron. The colloidal iron reagent of Rinehart and Abu'l Haj (1951) is made by dissolving 18.75 g of ferric chloride in 62.5 ml analar grade glycerine and then gradually 13.75 ml, 28% ammonia is added with constant stirring. This mixture is dialysed against double distilled water for five days with regular changes of the water. After staining in this solution sections were washed in distilled water. The sections were then flushed with a solution containing 0.02 M ferrocyanide and 0.14 M HCL for 15 minutes. Slides were washed well before treating with PAS stain.

Protein:

Mercury bromo-phenol blue (Hg BPB) was used to stain protein in the tissues. 1% mercuric chloride and 0.05% bromophenol blue in 2% aqueous acetic acid was preferred over 1% alcoholic bromophenol blue saturated with mercuric chloride. After staining for one hour, slides were rinsed in 0.5% acetic acid and directly transferred to tertiary butyl alcohol for restoring the pH to the neutral point in order to produce the blue form of the dye.

Nucleic acids:

Toluidine blue-methyl green-orange G method (Korson, 1951) has been adopted to stain ribonucleic acid (RNA). It was found that RNA is well stained with this method. Tissues fixed in cold Carnoy's fluid was used for this study. Parallel sections meant for control were treated with 10% perchloric acid at 80°C for five minutes only. Sections fixed in cold Carnoy were used to stain DNA with Schiff's reagent prepared as adopted by Gurr (1958). The sections were hydrolysed in N-HCL for 10 minutes at 60°C. Tissue was stained in Schiff's reagent for only 40 minutes. 1% aqueous light green was used as counter stain.

R E S U L T S

Carbohydrates:

When treated with periodic acid Schiff's reagent (PAS) under normal fed condition the outer membrane of the peritoneal layer, basement membrane and intercellular membrane of both free and nephric tubules, the striated border of the proximal region of free tubule and the muscle layer of crypto-nephric complex of Mylabris pustulata show strong positive reaction. However, under starved condition, these structures are only poorly stained (Plate XVII, Fig.1). In the case of Laccotrephes maculatus the basement membrane takes strong PAS stain in all stages.

Even at the end of 2 hours period after the commencement of feeding in the starved insect, proximal region of the free tubule of Mylabris pustulata shows only a weak reaction to PAS (Plate XVIII, Fig. 1), whereas the distal region exhibits a negative reaction. The muscle layer of crypto-nephric complex shows a positive reaction (Plate XVIII, Fig. 3). At this period a very weak PAS positive spot is observed near the nucleus in the distal region of the tubule.

The enlarged cells in both the regions of free tubules as well as, pronephric epithelium appearing at 6 hours period after feeding show PAS negative reaction (Plate XVII, Fig. 5). Upto 15 hours period, there is no change in the reaction of PAS either in the free tubule or in the nephric tubule; but the muscle layer of the crypto-nephric complex shows many strong PAS positive substances.

At 15 hours period many PAS positive granules appear in the cytoplasm which tend to accumulate as a dark patch near the nucleus of the cell in the distal region (Plate XVIII Fig.4 G). In the proximal region, however only the border takes up the stain (Plate XVII, Fig. 3). These granules disappear from the cell after 20 hours but in certain cells few granules can be traced even at 22 hours period (Plate XVII, Fig. 7). At 50 hours period of starvation the reaction of PAS stain as a whole become very weak (Plate XVII, Fig. 1).

At 2 hours period after the commencement of feeding in the previously starved Laccotrephes maculatus, PAS positive granules are accumulated towards the apical part of the cell within the first and second regions (Plate XXII, Fig. 1&2 G). In the third region the PAS positive granules are found scattered throughout the cytoplasm (Plate XXII, Fig. 2 G). At 4 hours period the third region shows no PAS positive

granules whereas, the border is PAS positive. But in the second region PAS positive granules are found to occupy the apical half of the central zone (Plate XXII, Fig. 3G). These granules are also found to accumulate in an irregular fashion in the apical half of the central zone of the first region (Plate XXII, Fig. 4 G). At 8 hours period the PAS positive granules are aggregated in the basal zone of the second region (Plate XXII, Fig. 5 G), but are mostly absent from the apical half of the central zone except for one or two patches of few granules each in some sections. In the third region during this period the granules appear again and are found scattered in the cytoplasm in the same manner as found at 2 hours period. At 10 hours period it is found that PAS positive granules are confined to one or two big patches where many granules are aggregated in the cytoplasm of the third region (Plate XXII, Fig. 6 G). The border zone of this region is strongly positive to PAS stain as is the case at 4 hours period. At the same time the apical border of the second region is occupied by these granules which tend to accumulate in this region.

At 12 hours period the phase resembles that of 6 hours period. Observation during the first 24 hours period reveal that these granules appear in a cyclic manner starting immediately after feeding and ending after 4-8 hours period.

After 10 days of continuous starvation, no PAS positive granules are observed.

Sections treated with acetic anhydride prove that these PAS positive substances are carbohydrate in nature. Toluidine blue method of Kramer and Windrum (1955) and that of Pears (1960) give a negative result for metachromatia. Similarly, the Alcian blue method of Steedman (1950) gives a negative result for acid mucopolysaccharide.

Combined dialysed iron and PAS stain (Ritter and Oleson, 1950) a modification of Hales method (1946) throws light on the nature of Carbohydrate contained in these tissues. The outer peritoneal membrane, the basement membrane of the free tubule and nephric tubule, the border of the proximal region of free tubule, the cell outlines of the muscle layer and pronephric epithelium of crypto-nephric complex are found to be positive to the mucoprotein except during the starved condition. Though much changes have not been observed in these parts of the cells many differentiation have been found in the ground cytoplasm of free and nephric tubules and pronephric epithelium of Mylabris pustulata at different periods.

At 2 hours period, after the commencement of feeding the cytoplasm of the cells of the free tubule is protein

positive in reaction while the basal zone of the cell which has many infoldings is mucoprotein positive. However, some cells as a whole are mucoprotein positive. After 4 hours period some cells are completely mucoprotein while others are protein in reaction. This is the case with the entire free tubule except the border zone of the proximal region which is strongly mucoprotein. In certain cases mucoprotein positive granules appear to leak out from the cell border (Plate XVII, Fig. 2). In the case of crypto-nephric complex the nephric tubule is weak to mucoprotein reaction while few strongly mucoprotein positive spots are found in the tubule cytoplasm (Plate XVII, Fig. 2). Pronephric epithelium is also protein positive in nature.

The enlarged cells after 6 hours period of feeding show negative reaction to mucoprotein. But many nucleo-protein positive substances are found in the cytoplasm of the cell (Plate XVI, Fig. 3, NP). The cytoplasm of few normal cells show protein as well as, mucoprotein reaction. Nephric tubule as a whole is protein positive but pronephric epithelium shows nucleo-protein positive substances in the cytoplasm (Plate XVI, Fig. 4, NP). After 8 hours period all the cells of different regions become completely mucoprotein positive except for the rare occurrence of one or two spots

of pale blue colour indicating the presence of some protein in the cytoplasm but nephric tubule shows only few granules of mucoprotein positive substance (Plate XVI, Fig. 2, MP).

After 10 hours period whole cytoplasm of the cell in different regions of free tubule becomes protein positive in reaction. In the case of nephric tubule the stain appears in the form of shades of both pale blue and red indicating that the cytoplasm is positive to both mucoprotein and protein. At 12 hours period the proximal nephric tubules loss its mucoprotein nature and become exclusively protein in nature.

At 15 hours period the cells of the proximal region show moderate mucoprotein reaction while the border is strongly positive in reaction. In the distal region both types of cells with positive protein as well as, mucoprotein are present. There are also cells displaying both protein and mucoprotein reaction. The granules which are positive to PAS stains are found to be mucoprotein positive as well (Plate XVII, Fig. 6, G).

After 20 hours period granules of mucoprotein begin to disappear. The proximal region is completely protein positive in nature. A sudden change in the staining reaction

of the pronephric epithelium takes place during this period. It shows positive reaction to the mucoprotein. There are many deeply mucoprotein stained granules as it is the case at 4 hours period, in the cytoplasm of nephric tubules.

Upto 46 hours period three types of cells are distinguishable in the free tubule irrespective of regional differentiations. Some cells are deeply protein positive while others are mucoprotein positive in reaction. The third type of cells exhibit both mucoprotein and protein reaction. But at 27 hours period pronephric epithelium becomes protein positive in nature. The nephric tubule is weakly mucoprotein in reaction. This continues upto 44 hours period. At 46 hours period nephric tubules show both protein and mucoprotein positive reaction.

At 48 hours period the cells of the distal region of free tubule become protein positive. After 50 hours period when the big cells appear the cells show no mucoprotein substances in it (Plate XVI, Fig.5). Similarly, pronephric epithelium shows no mucoprotein. At 96 hours period of continued starvation (when the insects become inactive) the staining reaction as a whole become very weak (Plate XVI, Fig. 1 & 6).

In the case of Laccotrephes maculatus, the basement membrane and the border zone take mucoprotein stain but the

cytoplasm shows no staining reaction (Plate XXII, Fig. 8) except for the PAS positive granules which are found to be mucoprotein positive as well.

When the sections of the free tubules and also the crypto-nephric complex of M. pustulata after 15 hours period of feeding and the tubules of L. maculatus after varying periods of feeding are incubated with human saliva and stained with PAS as well as, combined dialysed iron PAS stain, the granules which were earlier found positive to these stains show negative reaction (Plate XVIII, Fig. 6 and Plate XXII, Fig. 7). These granules are stained with Best's Carmine proving their glycogen nature (Plate XVIII, Fig. 5, G and Plate XXIII, Fig. 1, G).

Sections of the tubules of both M. pustulata and L. maculatus are negative to starch as shown with Lugol's iodine.

Protein:

In the case of M. pustulata the basement membrane of the entire free tubule is strongly positive to HgBPB. The cytoplasm is strong protein in reaction in all the regions of tubule (Plate XIX, Fig. 2). Same is the case with the nephric tubule and pronephric epithelium (Plate XIX, Fig. 4).

The enlarged cells at 8 hours period are HgBPB positive in reaction. At 15 hours period the cells show a positive HgBPB reaction in the basal and proximal half of the central zone of the cytoplasm while the distal half of the zone shows negative reaction (Plate XIX, Fig. 5). After 50 hours period the staining reaction is very weak in all the regions of tubule (Plate XXI, Fig. 8) and also in the crypto-nephric complex (Plate XIX, Fig. 3).

The basement membrane, border zone and ground cytoplasm of the malpighian tubules of Laccotrephes maculatus is HgBPB positive in reaction (Plate XIX, Fig. 7). At 4 hours period many HgBPB positive granules are found in the third region of the tubule (Plate XIX, Fig. 8, PPS). In the starved condition the reaction to HgBPB becomes very weak (Plate XIX, Fig. 6).

Nucleic acids (DNA and RNA):

The Feulgen positive substances appear in the nucleus as two or three granules in the free tubule, pronephric epithelium and nephric tubule of M. pustulata (Plate XX, Fig. 1&2, DPS).

Upto 6 hours period the intensity of the staining is very weak. The enlarged cells of free tubule at 8 hours

period show a negative reaction (Plate XX, Fig. 3) while the nuclei of pronephric epithelium and nephric tubule of crypto-nephric complex display positive reaction to Feulgen stain (Plate XX, Fig. 4, DPS). Although the Feulgen reaction becomes strong after 8 hours period it begins to weaken at 46 hours period when the nuclei are filled with dark granules (Plate XX, Fig. 5&6, GN).

In the case of L. maculatus, Feulgen positive reaction is observed in the form of small stained granules inside the nuclei of different regions (Plate XXIII, Fig. 3, DPS). After continued starvation these granules stain very weakly (Plate XXIII, Fig. 2). No other changes have been observed.

The RNA positive stain in the case of Mylabris pustulata is found in the form of two or three spots inside the nucleus and small granules inside the cytoplasm of free tubules (Plate XX, Fig. 7&8, RNS) as well as, in the nuclei of crypto-nephric complex (Plate XXI, Fig. 2, RNS). Many changes are found with respect to RNA staining reaction during the course of experimental study.

In the case of Mylabris, at the time of starvation cytoplasm of proximal region shows many minute granules accumulated in close proximity of the border zone (Plate XXI,

Fig. 1). Nuclei are found to contain two or three big granules throughout the entire length of the free tubule.

The enlarged cells of free tubule at 6 hours period and also pronephric epithelium show active RNA positive staining (Plate XXI, Fig. 3, RNS). There are many oval shaped bodies filled with RNA positive granules in the pronephric epithelium. At the same time nuclei of nephric tubule are poor in reaction to RNA stain (Plate XXI, Fig.4, RNS). But at 8 hours period nuclei of nephric tubule show strong reaction (Plate XXI, Fig. 2, RNS).

After 46 hours period there is considerable reduction in the RNA staining reaction in the distal region of the free tubule (Plate XXI, Fig. 5) and throughout the crypto-nephric complex except in the region where pronephric epithelium comes in contact with nephric tubule (Plate XXI, Fig. 6). After 50 hours period nucleus of the leptophragma also exhibit strong RNA positive reaction (Plate XXI, Fig. 7, RNS).

In the case of L. maculatus, under starved condition RNA is limited to two or three spots in the nuclei of all the regions (Plate XXIII, Fig. 4, RNS). At 4 hours period of starvation the nuclei are filled with many RNA positive granules (Plate XXIII, Fig. 5, RNS). The third region also

show many RNA positive granules in the cytoplasm (Plate XXIII, Fig. 6, RNS). At 6 hours period RNA positive granules disappear from the cytoplasm of the third region but the nucleus and nucleolus show strong positive reaction. At 8 hours period again the reaction is similar to that at 4 hours period. This cycle continues upto 96 hours period only, after which the RNA reaction is found to be very weak.

Non stained granules:

When the tissues of M. pustulata are treated with PAS, modified Hale's method and Feulgen, many granules are found to occupy the nuclei both in free tubules and in the crypto-nephric complex (Plate XVI, Fig. 8 and 6, NG). No specific region or period could be observed for the presence of granules. But they are found more in the starved condition. In this respect even the neighbouring cells show some difference. The nature and the distribution of these granules differ greatly. In certain instances these granules accumulate in the centre of nuclei, rarely along the periphery of nucleus. Mostly they are found to occupy the whole space of the nuclei so that the latter appear black in colour after the treatment with the stain. In certain cells such granules are found in cytoplasm as well (Plate XVI, Fig. 7).

When the sections are stained with Feulgen stains crystal like granules appeared in the nucleus of the free and nephric tubule of starved M. pustulata (Plate XX, Fig. 5&6, GN).

In the case of L. maculatus non-stained crystal like granules are found only in the cytoplasm of the third region of the tubule. Whereas, in M. pustulata no such granules are observed either in nucleus or in the cytoplasm. In the third region of L. maculatus these granules appear at four hours period after feeding whereas, during the period of starvation these granules disappear altogether.

D I S C U S S I O N

Carbohydrates and protein:

Both in the case of Laccotrephes maculatus and Mylabris pustulata the outer coverings of the malpighian tubules are strongly positive to periodic acid Schiff (PAS) test and this reaction is not weakened by incubating the sections with saliva before performing the PAS test. Similar reaction has been observed with the border zone of the proximal region of the malpighian tubule, the basement membrane of the pronephric epithelium, muscle layer and nephric tubule of M. pustulata. The method of Kramer and Windrum (1955) as well as, that of Pears (1960) to stain metachromatia with Toluidine blue have given a negative result. Since the Alcian blue reaction which is considered to be an indicative of acid polysaccharide shows a negative reaction and the latter usually donot give a positive PAS reaction (Pears, 1960) it is evident that these PAS positive substances are not acid polysaccharide. These results suggest that the above mentioned structures of the malpighian tubule contain a carbohydrate which is neither glycogen nor acid mucopolysaccharide.

Histochemical studies have indicated that the basement membrane of the gut, fat body, sarcolemma and ovary contain neutral mucopolysaccharide and that they are similar to the neural lamella of insects (Baccetti, 1955, 1956; Pipa and Cook, 1958; Bonhag and Arnold, 1961). The studies on the neural lamella of Periplaneta americana (Richards and Schneider, 1958; Hess, 1958 a & b, Ashhurst, 1961) suggest a collagen type protein together with neutral polysaccharides in the neural lamella. The mercury bromophenol blue (Bonhag, 1955) and Hale's modified method (combined dialysed iron and PAS stain) of Ritter and Oleson (1950) show affirmatively that these structures are positive to protein and mucoprotein, respectively. Distribution of mucocomplexes in insects has been studied by Frenzel (1886), Ichikawa (1931), Sundman and King (1964). Von Dehn (1933) and Wigglesworth (1948) are of the opinion that mucoid materials are absent from insects especially in the gut. Day (1949) has also reported that mucoid materials seem to be of less frequent occurrence in insects. But now it is confirmed that mucocomplexes actually occur in several insect tissues. Very small quantities of histochemically characterised mucocomplexes of various categories are produced by the malpighian tubules of a number of insect species belonging to several orders (Gabe, 1962). In

Cercopid larvae although the production of mucoprotein has been attributed to the malpighian tubules (Pesson, 1956). Recently, Marshall (1966) has shown that it is actually acid mucopolysaccharide since it is not associated with detectable protein.

Meyer (1938) considers that neutral mucopolysaccharides from animal sources always occur in firm combination with proteins and he defines mucoproteins as substances in which hexosamine containing polysaccharide are found in firm chemical union with peptide. Lison (1953) observed that dialysed iron method, stains the nucleoprotein and other proteins and Lillie (1954) agrees that it is not selective for acid polysaccharides. Davies (1952) has found that a positive reaction is given by fibrin, gelatin, casein and even by peptone. Braden (1955) confirms these results. Protein nature of these PAS positive substances is evident from its positive reaction to the HgBPB which has been adapted as a general stain for protein by Mazia et al. (1953). This method is also employed by Bonhag (1955) for investigating the composition of ovary of the milk weed bug, Oncopeltes fasciatus.

The PAS positive granules which are present near the nucleus in the distal region of malpighian tubules of

Mylabris pustulata at 15 hours period after feeding and the PAS positive substance, found in the muscle layer of crypto-nephric complex disappear completely when treated with saliva prior to staining with PAS. These granules although positive to Hale's modified method of Ritter and Oleson (1950) are not stained when incubated with saliva. The action of Ptyalin (salivary amylase) at room temperature for 30 minutes or less is considered sufficient to remove glycogen from paraffin sections irrespective of the fixative employed (Pears, 1960). Same is the case with the PAS positive granules found in the different regions of the tubules of Laccotrephes maculatus. Glycogen nature of these granules are confirmed from their positive reaction to the Best's Carmine stain. The distribution of glycogen is known in the digestive tracts of insects and its presence has been indicated in the malpighian tubules of several insects (Gabe, 1962).

Both in M. pustulata and L. maculatus the glycogen granules, found in the malpighian tubules and also in the muscle layer of crypto-nephric complex disappear when insects are starved. Soon after feeding (at 15 hours period in the case of M. pustulata and at 2 hours period in the case of L. maculatus) these granules appear in the

tubule. In the newly moulted 4th stage mosquito larva it has been reported that glycogen is abundant in the muscles; but after 12 days of continuous starvation glycogen is reduced to faint traces. In a similar manner immediately after providing a carbohydrate meal to the fully starved larva, glycogen appears in the form of granules within the cells of malpighian tubules and also in scattered manner in the sarcoplasm of muscles (Wigglesworth, 1942).

Experimental studies show a fall in the glycogen content in starved insects. In the case of starved mealworm the glycogen content falls from 2.04% of wet weight to 0.68% in a week (Mellanby, 1932). In Odonata it falls from 0.2% to zero (Slowtzoff, 1904, 1905, 1909). In the larva of Popilla japonica, starved for four weeks, 80% of the glycogen is consumed (Ludwig and Wugmeister, 1953). Glycogen is used concomitantly in Drosophila during starvation (Wigglesworth, 1949). Since these glycogen granules appear immediately after feeding and are found throughout in the normally fed insects, it is suggested that glycogen is kept as reserve material to be used when required. The arguments put forth by Wigglesworth (1942) that these granules appearing in the malpighian tubules are

derived from the gut contents which occasionally enter the tubule appear to be untenable. The studies on the malpighian tubules of blowfly, Calliphora erythrocephala (Berridge, 1966) indicate the presence of a classical glycolytic-kreb cycle pathway for the metabolism of carbohydrates in tubule cells.

It is surprising to observe that these granules which are PAS Best's carmine positive are non-resistant to saliva and show a cyclical activity connected with the functional physiology of malpighian tubules of Laccotrephes maculatus. It can not be satisfactority explained at present since the functional differences observed in histochemistry are more striking, and are complicated than what have been revealed from histological studies as is the case with the digestive tracts of insects (Waterhouse, 1957). It is also difficult to correlate the present observations with the available reports on glycogen because many differences are recorded in the same tissue in related insects (Gouranton, 1968b).

The ground substance of the cell is very weak in both M. pustulata and L. maculatus to PAS stain during starved condition but it is moderate in the fed insects. Cytoplasm

in both insects is strongly positive to HgBPB reaction. Similarly, modified Hale's method of Ritter and Oleson (1950) shows a positive reaction to protein; but in the case of M. pustulata reaction for mucoprotein as well as, protein are noticed in the same cells and also in separate cells. Since different reactions are observed both in the free and nephric tubules and the pronephric epithelium it clearly indicates that mucocomplex also play an important role in the physiology of malpighian tubules. During starved condition mucoprotein is stained more densely in the distal part of the nephric tubule and it also appears in pronephric epithelium which shows only protein positive reaction in normal feeding insects. Recently Khalil (1971) has shown by means of incorporation of tritiated tyrosine that mucopolysaccharide plays an important role in the excretory physiology of certain ticks. Hibiscus rosachinensis the common food of M. pustulata contains a huge quantity of mucoidal substances. It appears, therefore, reasonable to suggest that the mucoid substance, abundant in the food may also be present in good quantity in the haemolymph which is utilised for the physiological functioning of the tubule. A mucopolysaccharide is also thought to provide anion groups to balance the large excess of cations in the extracellular fluid of cockroach abdominal ganglia (Treherne, 1962). The

urine of Dysdercus contains mucopolysaccharide which according to Berridge (1965) consists of free anion groups and so contribute to the large anion deficit. A glycoprotein has also been reported from the excreta of three species of mosquitoes (Irreverre and Terzian, 1959) although the possibility that it is derived from the gut is not ruled out. Glycoproteins are distinguished from mucoprotein on the arbitrary basis of their hexosamine content of less than 4%. This division is suggested by Meyer (1938) who gives only rough histochemical distinctions (Pears, 1960). In Locusta, Martoja (1959) has reported the presence of mucus cells among the normal cells of the tubule. From the present study it is evident that not only are the malpighian tubules responsible for the production of urine or the physical removal of waste materials but they also play a significant role in the excretory metabolism.

Nucleic acids (DNA and RNA):

Feulgen reaction for DNA has been extensively utilized to ascertain the distribution of deoxy-ribonucleic acid in various types of cells (Pears, 1960). Though DNA appears to play only little role in the tubule physiology, there is a decrease in the intensity of staining nature of DNA after starvation for three days and six days in the case

of M. pustulata and L. maculatus, respectively. In rat liver (Lagerstedt, 1949) and in Euglena (Melkoff and Bucton, 1964) due to starvations the DNA becomes diffusely distributed throughout the nucleus.

Both in M. pustulata and L. maculatus malpighian tubule cells show a higher RNA content when treated with Toluidine blue-Methylgreen-Orange G method of Korson (1951). However, when the sections are treated with 10% perchloric acid (Gurr, 1958) the RNA is extracted out and the sections show a negative result. It is interesting to notice under experimental conditions certain enlarged cells both in the free tubule and pronephric epithelium with many RNA positive substances.

Enhanced protein synthesis in the tissue is correlated with an increased level of RNA (Gilmour, 1961). Takeyama et al. (1958) have shown a cyclical role of RNA in fibrin synthesis in the silk glands of Bombyx. The association of high level local concentration of nucleic acids with protein synthesis (Brachet, 1942; Casperson, 1947; Davidson, 1949) is apparent from studies of pancreas, salivary glands, hair follicles and the basal layer of epidermis, eggs and embryonic tissues in vertebrates and regenerating tissues as well as, silk glands of invertebrates (Bradfield, 1950).

Thomas and Nation (1966) have suggested that in Periplaneta americana the low levels of body protein and decreased synthesis of tissue protein may be consequence of fall in RNA synthesis. Following a natural or experimentally produced rise in molting hormone titre, malpighian tubule cells of Drosophila hydei display a change in the chromosomal puffing pattern (Berendes and Williart, 1971). Similarly, topical application of juvenile hormone on pharate adults causes the formation of a specific puffs within one hour in the malpighian tubule chromosomes (Holderegger and Lezzi, 1972). Clever and Karlson (1960) have observed in the larvae of Chironomus tentans that injected ecdysone induces chromosomal puffs which are demonstrated by autoradiography as the sites of RNA synthesis. The increase in RNA in the digestive cells is no doubt connected with synthesis of enzymes utilized in intracellular digestion (Rosen, 1941). Many enzymes are synthesized in the malpighian tubules for utilization in different physiological activities. Mazzi and Baccetti (1956) have found phosphatase activity in both the proximal and distal portions of the tubules in Donus crinitus. In Acrida bicolor, Blaps gibba, Apis mellifera and Mantis religiosa (Mazzi and Baccetti, 1957b) alkaline phosphatase and acid phosphatase, lipase and succinic dehydrogenase have been

reported from different regions of the tubule. A fairly strong dipeptidase is also present in the tubules of carabid beetles, grasshoppers and the cockroach (Schlottke, 1937a,b). Auclair (1959) has studied the amino acid oxidases present in the malpighian tubules of Periplaneta americana, Galleria mellonella, Blatella germanica and Oncopeltes fasciatus. An intercellular deaminase acting upon higher peptides is reported in the tissues of blowfly larva (Brown and Farber, 1936). Xanthine dehydrogenase is found in the malpighian tubules of Bombyx mori (Hayashi, 1961). Similarly, uricase is reported to be present in the tubules of Dysdercus fasciatus (Berridge, 1965). Transaminases have been found in the tubules of Schistocerca gregaria (Kilby and Neville, 1957).

RNA positive substances in M. pustulata during fed condition are found in the cytoplasm of the distal part of the tubule whereas, in the starved condition these substances are concentrated in the cytoplasm of the proximal region. Similarly, during starved condition the nuclei of lepto-phragma are fully packed with RNA positive materials. In the case of L. maculatus, it appears in the cytoplasm of the third region after feeding. It is, therefore, suggested that RNA plays an active role in the cellular physiology of the tubule. Active transport (Ramsay, 1952, 1953b, 1955b)

and reabsorption (Wigglesworth, 1931; Bahadur, 1961,1964; Wessing and Eichelberg, 1969) take place in the tubule and secretion as well as, reabsorption are performed at the expense of energy (Berridge and Gupta, 1968). The higher RNA content showsthat the cells are actively engaged in secretary function (Sunner, 1965). But an increase in the RNA positive substance in the enlarged cells within the free tubule cells and also in the pronephric epithelium of M. pustulata may be a compensatory device to cope with the little quantity of RNA found in the nuclei. On the other hand L. maculatus has a higher RNA content in the nucleus besides the strongly RNA positive nucleolus. The malpighian tubules of L. maculatus differ from those of M. pustulata in that in the former it takes several days rather than three days for the changes to occur. It seems as one can expect that L. maculatus is adapted for living without food for long periods.

Non stained granules:

Many non stained refractile granules are found in both free and nephric tubules and also pronephric epithelium of M. pustulata and L. maculatus. In the case of M. pustulata the granules found in the nucleus vary in shape, size and distribution. Since these granules are found in the nuclei, and in their presence nucleoprotein, DNA, and protein are not stained with Hale's modified stain, Feulgen, and HgBPB,

it appears that these granules may be the product of nuclear katabolism. In the case of L. maculatus the nuclei donot show such granules in normal fed insects. There are many reports of various substances present in the malpighian tubules although no particular significance can be attached to their presence (Craig, 1960). It is known that pteridines and other fluorescing substances are found in the tubules. An experimental survey of fluoresence of the malpighian tubules of a number of species of Blattidae has been made by Willis and Roth (1956), but no information has been given as to the nature of substances present. In most insects the granules present in the cytoplasm of the tubules cells are not the same as the excretory granules found in the tubule lumen (Wigglesworth, 1965). Recently, Lhonore (1970) has reported the presence of light brown pigment globules inside and outside the nuclei of Gryllotalpa. Similarly, cytophysiological studies on Gryllotalpa gryllotalpa (Lhonore, 1971) have proved that yellow tubules excrete spheroids of phosphates of calcium, potassium, magnesium etc., which crystalise in the cytoplasm. Non-stained granules found in the third region of the tubule of L. maculatus and different regions of the tubule cytoplasm of the M. pustulata may fall in this category.

BIOCHEMISTRY OF THE MALPIGHIAN TUBULES

INTRODUCTION

Instead of making conclusions from experiments in which whole insects are used, recently the biochemical studies on insects have been switched on to individual organs and that the findings of insect biochemists are now comparable to those of mammalian biochemists. The study of insect metabolism in relation to nutritional state is of more recent date. The pertinent literature on insect metabolism has been reviewed by Gilmour (1961) and Chefurka (1965). Since in most studies homogenates of whole insects have been used the properties of individual organs are very little known (Chen, 1966). Consequently, the observations available do not help to understand the true metabolic interrelationships between different organ systems. A large gap exists, between nutritional experiments and their interpretation in terms of metabolic events in individual organs. Two insects namely Mylabris pustulata and Laccotrephes maculatus have been selected to provide some informations on this aspect.

Quite good numer of works are available on determinations of total nitrogen content of insects (Fricker, 1885; Pagnoul, 1895; Zaitschek, 1904; Slowtzoff, 1909; Inouye, 1912; Abderhalden, 1923; Jarvis, 1923; Dingler, 1927).

Similarly the effect of starvation on body composition is reported in Tenebrio molitor and other insects (Mellanby, 1932; Wigglesworth, 1942).

Studies on nitrogenous end products have also been carried out as an aid to the study of metabolism (Brown, 1936; Powning, 1953; Nation and Patton, 1961). It has been found that the type of food taken by the insect determines the quantity of nitrogenous end products in Anthronomous grandis (Mitlin et al., 1964). For example the excretion of ammonia by the larva of Lucilia sericata is closely dependent on protein food (Brown, 1938). Similarly, in muscid flies pure protein diet results in excretion of ammonia and uric acid about twice as great as the normal diet (Brown, 1936). Feeding in Aeshna cyanea (Staddon, 1959) is followed by a large temporary increase in the amount of ammonia excreted. The total nitrogen content of urine in the fifth instar nymph of Dysdercus fasciatus (Berridge, 1965) increases slightly towards the end of post excretory phase. In the mature larva of Galleria mellonella the haemolymph purine nitrogen and excretory purine nitrogen is found almost the same even though the insects were fed on two different media (Nation and Thomas, 1965). These evidences indicate towards the

importance of nitrogen in the metabolism of insects during feeding as well as, starvation. But hardly any of the papers dealing with the chemical composition of insect excreta or tissue make any attempt to interlink them with the normal fed insects or with starved ones. Since malpighian tubules are demonstrated as the organs of elimination of nitrogenous end products, some preliminary studies have been undertaken to investigate the role of total nitrogen concentration in the tubules in relation to feeding and starvation.

Protein concentration in insect haemolymph is similar to that of blood of man but generally higher than that of other vertebrates (Florkin and Jeuniaux, 1964). Though several works on the determinations of the protein percentage in whole insects are available (Slowtzoff, 1905, 1909), determination of protein in individual organs are meagre excepting the reproductive organs and fat bodies of adult (Shigematsu, 1960; Loughton, 1965; Price and Bosman, 1966) and distribution of protein in the blood during the development (Telfer, 1954, 1960; Laufer, 1960; Orr, 1964a,b; Loughton, 1965). Increase in the haemolymph concentration of proteins has been related to the increase in the fat body in Schistocerca (Hill, 1965). Similarly, sequestration and storage of haemolymph proteins by fat cells during larval/pupal transformation has been demonstrated in Calpodes ethlius (Locke

and Collins, 1968) in Pieris brassicae (Chippendale and Kilby, 1969) and Galleria mellonella (Collins and Downe, 1970). Locke and Collins (1968) and Tobe and Loughton (1969) have shown that in Locusta during the last nymphal instar labelled haemolymph proteins are taken by the fat body and other tissues.

Although statistically no significant difference is observed in the total protein concentration in the haemolymph of the larva of P. brassicae fed on cabbage and of those fed on artificial medium (Van der Geest, 1968), Orr (1964a) has reported a striking rise in the blood protein on the third day after feeding in blowfly, Phormia regina. Similarly, in the housefly a substantial increase in haemolymph protein occurred after one day of milk feeding (Bodnaryk and Morrison, 1966). Starvation has also been demonstrated to bring a significant reduction in the total protein. It has been clearly shown in Phormia by the recent work of Orr (1964a,b) that during enforced starvation it may drop to extreme low levels. The same is true for both Celerio euphorbiae (Heller and Moklowska, 1930) and Sialis lutaria (Beadle and Shaw, 1950). Therefore, it is desirable to have some observations on the protein concentration in insect malpighian tubules to know its role on tubule physiology.

From the data available on the organ Lipids of animals such as rat, it may be concluded that wide variation exist both in the nature and distribution of lipids in different parts of the same animal species (Shorland, 1960). This has established the importance of lipids in tissues and organs in relation to structure and function (Green, 1959) and has brought about a sudden burst of interest on the occurrence and function of phospholipids.

Although numerous analyses of phospholipids of vertebrates have been made, similar studies on insects are relatively recent (Beiber et al., 1961; Fast and Brown, 1962; Crone and Bridges, 1963; Fast, 1964; Kamienski, et al., 1965; Khan and Hodgson, 1967). Changes in phospholipids during the development have been given more attention. Bridges and Cox (1962) has reported that there is little change in the lipid phosphorus during development of Musca. Similar reports have been made in Phormia regina (Bieber et al., 1961). Except the work of Crone (1964) Taylor and Hodgson (1964) Khan and Hodgson (1967) Thomas and Gilbert (1967) others are on phospholipid composition of whole insect.

It is reasonable to believe that the composition of individual organs might be quite different from that of the whole organism. For example sarcosomes of housefly contain

more phosphatidylethanolamine and less phosphatidylcholine than the same in whole flies (Crone, 1964). The presence of lysophatides has also been reported in the fat body of Sarcophaga bullata (Allen and Newbergh, 1965). These studies indicate that differences in the phospholipids may exist at the organ level. Recently, Crone (1964), has indicated that change in the diet may alter the phospholipid composition of housefly.

As the phospholipids occur primarily as components of biological membranes, their function and metabolism may be better understood if their concentration in an organ under various physiological conditions is known. Malpighian tubules are organs where biological membranes serve an important role in active transport (Ramsay, 1953b) secretion and reabsorption (Wigglesworth, 1931; Berkaloff, 1960; Srivastava, 1962; Bahadur, 1964). It is, therefore, of interest to examine the phospholipid concentration in the malpighian tubules under normal and experimental conditions so as to correlate its functional significance.

MATERIAL AND METHODS

Mylabris pustulata and Laccotrephes maculatus of same age and reared under similar conditions were used for all biochemical estimations. During the present study only male insects have been used for all biochemical estimations.

Experimental procedure:

Male insects were removed from the rearing trough and kept in separate jars each of which containing 8-10 insects. M. pustulata were starved continuously for three days whereas, L. maculatus were starved for 10 days. Later they were allowed a full meal. M. pustulata were fed on the flowers of Hibiscus rosachinensis whereas, L. maculatus were fed on mosquito larvae. After a full meal they were removed from feeding trough and kept in separate jars without food. At intervals the insects were sacrificed to examine the tissue and blood.

Preparation of blood sample:

Since handling or anaesthesization of M. pustulata produced reflex bleeding the insects were anaesthesized very slowly by passing ether vapour into a big jar containing the insects. L. maculatus on the other hand were directly

anesthesized in ether vapour. After anaesthesization, M. pustulata and L. maculatus were taped separately to a clean glass slide. A puncture was made in the cervix region and jentle pressure was applied on the abdomen. The haemolymph that oozed out was collected in a micro-pipette and kept under refregeration until the time of estimation. Atleast 12-15 M. pustulata were needed to collect required quantity of blood. In the case of L. maculatus 30-40 insects were used to collect the desired quantity of blood.

Preparation of tissue:

Insects after anaesthesization were dissected under binocular microscope in insect ringer's solution. The tissue was removed from the body as quickly as possible and rinsed twice in Ringer's solution to wash out the haemolymph. Later it was transferred to tubes containing a few drops of Ringer's solution and kept under refregeration till use. Malpighian tubules of 8 M. pustulata were pooled to get the required tissue while in the case of L. maculatus, 15 insects were needed.

Determination of the food consumed and excreta voided:

After starvation period the insects were weighed and the body weight recorded. Similarly, after a full meal the

insects were weighed and the difference in weight was taken as the approximate weight of the food consumed by the insect.

M. pustulata were kept on previously weighed filter, paper. At intervals the paper was removed and weighed. Difference in weights was taken as the weight of excreta. In the case of L. maculatus the animal was transferred immediately after feeding to a small polythene beaker containing distilled water, the weight of which was known. At intervals insects were removed carefully and the beaker with water was weighed, and the difference in the weight was taken as weight of excreta.

For the determination of moisture content of Hibiscus rosachinensis 3 flowers were weighed and kept in previously weighed beaker and placed in an oven running at 100°C for about 28-32 hours (A.O.A.C., 1960) till it became completely dry. The whole process was repeated until a constant weight was obtained. The loss of weight gave the weight of water content from which it was calculated that Hibiscus flower contains 70.6% water.

Nitrogen and protein:

Nitrogen was estimated by a slight modification of Wong's (1923) micro-kjeldhal method. In the case of malpighian tubules of normal M. pustulata only 30 mg of sample

was enough for estimation whereas, in the case of experimental M. pustulata and both normal and experimental L. maculatus 100 mg of tissue and 1 ml of blood was required for estimation. 100 mg of faecal matter was used to determine the nitrogen present in the excreta. The sample was digested in 5 ml of nitrogen free 1:1 sulphuric acid using potassium persulphate as oxidising agent. During digestion in sulphuric acid all the nitrogenous materials get converted into ammonium sulphate. The digested sample was made to 50 ml with distilled water. A known aliquot of this solution was nesselerised. The colour developed was read against a blank in spectronic 20 at 480 mu.

A calibration curve of ammonium sulphate was prepared by taking readings of series of solutions of different dilutions which always contained a known concentration of nitrogen. The reading obtained for the unknown solution was read against the ammonium sulphate calibration curve. This gives direct reading of total nitrogen present in the sample. The amount of total nitrogen thus obtained is multiplied by the conventional protein factor 6.25 to obtain the protein value (Hawk, Osser and Summerson, 1954).

Phospholipid:

Total phospholipid was determined by the method of Bartlet (1959) as modified by Marinetti (1962). For direct determination, 100 mg of tissue in the case of malpighian tubule and 2 ml in the case of blood was used. To the blood sample and the homogenised tissue sample, 5 ml of freshly prepared 10% trichloroacetic acid was added and allowed to stand for a few minutes and centrifuged at 3000 rpm for 15 minutes. The supernatant was decanted and the tubes were inverted until practically all supernatant has been removed. The contents of the above centrifuge tubes were digested in digestion unit with 1 ml of 70% perchloric acid for 25-30 minutes. On cooling, 7 ml of double distilled water and 1.5 ml of 2.5% ammonium molybdate (W/V) were added and mixed well. Finally 0.2 ml of 0.25% aminonaphathol sulphonic acid reagent was added. The tubes were treated in a boiling water bath for exactly 7 minutes, cooled and the colour was read at a wave length of 830 mu against a blank.

Calibration curve of monopotassium dihydrogen phosphate was prepared by taking readings of series of solutions of different dilutions which always contained a known amount of phospholipid phosphorus. The total phospholipid for the unknown sample was calculated from the standard curve. The phospholipid values were obtained by multiplying the phospholipid phosphorus by a factor of 25.

R E S U L T S

In preliminary experiments it has been observed that L. maculatus after a full meal can live for a longer time without food but in the case of M. pustulata which feeds more or less continuously survive but a few days when deprived of food. On the fourth day the insect either dies or become inactive.

Plate XIII, Graph E & F show the reduction in weight at the time of starvation. The difference in the weight of insect before and immediately after feeding gives the approximate weight of the food taken. However, it is difficult to calculate the amount of food taken by M. pustulata due to the fact that there is an excretory phase immediately on the onset of feeding. Usually it feeds continuously for 8-10 hours. At 4 hours period, after the commencement of feeding it starts defecating. So the loss of weight due to defecation is also taken into account in calculating the amount of food consumed by the insect. It is estimated that M. pustulata consumes as much as 0.71-0.73 g of food whereas, L. maculatus takes at a full meal 3.6-4.8 mg of food per insect. The weight of the body falls down gradually during

the starvation period both in M. pustulata and L. maculatus. But the weight of insects fed at regular intervals remained more or less constant.

During the first excretory phase M. pustulata voids less excreta in comparison to the excreta voided at the second excretory phase i.e., at 24 hours period, after a full meal. After the second phase of excretion there is a gradual decrease in the amount of excreta passed out during the period of starvation. Since no weighable amount of excreta could be found at short intervals after the first day in the normally fed M. pustulata, weight of excreta was calculated at regular intervals of 2 days. Data clearly suggest that the weight of excreta in the case of normally fed insects is more or less constant though some fluctuations are seen at certain periods (Plate XIII, Graph D). Similarly, the amount of excreta voided is more or less constant in the normally fed L. maculatus (Plate XIII, Graph C). On the second and fourth day periods after a full meal, L. maculatus under experimental condition voids a higher quantity of excreta, while the amount of excreta decreases gradually during the period of starvation. A peak in the excretory phase is observed only when both the insects are experimentally fed. In the normal fed insects the amount of excreta

varies from 0.53-0.64 g/insect in M. pustulata and 1.6-1.7 mg/insect in L. maculatus.

Nitrogen, protein and phospholipids in the blood and malpighian tubules:

Plate XIV, Graph A,B,C,D,E,F and Plate XV, Graph C, D, E show that the concentration of the total nitrogen, protein and phospholipids in the case of normally feeding M. pustulata and L. maculatus are more or less constant both in the blood and malpighian tubules with slight fluctuations at certain periods. In M. pustulata the concentration of total nitrogen, protein, and phospholipids vary from 0.256-0.324 mg/100 mg, 1.6 to 2.02 mg/100 mg and 0.39-0.43 mg/100 mg respectively, in the tubule and 4.36-5.23 mg/100 ml, 27.25-32.67 mg/100 ml, 14.2-16.2 mg/100 ml respectively in the blood. Similarly, in the normal L. maculatus nitrogen, protein and phospholipids vary from 0.015-0.022 mg/100 mg, 0.093-0.137 mg/100 mg and 0.34-0.42 mg/100 mg respectively, in the tubule and 2.00-2.72 mg/100 ml, 12.49-17 mg/100 ml and 12.4-14.1 mg/100 ml respectively in the blood.

During the period of starvation the total nitrogen content of malpighian tubules as well as, blood decreases in M. pustulata and L. maculatus though there is a little

gain immediately after feeding. Same is the case with the total protein and phospholipid of tubule and blood.

In the case of M. pustulata the total nitrogen and protein increases after the intake of food in the previously starved insects. The level increases from 8 hours period to 32 hours period in tubule as well as, blood. After this period the level of protein and nitrogen in the tissue of the tubule as well as, blood fall gradually during starvation (Plate XIV, Graph, B,C). The phospholipid in the blood of M. pustulata shows an increase at 12 hours and 24 hours period when compared with those of 8 hours and 16 hours periods. But this level gradually falls down after 24 hours period during starvation. At 72 hours period it shows an increase in the phospholipid level of the blood than that of 52 hours period (Plate XIV, Graph E,F). In the tubule homogenate the phospholipid level increases after feeding upto 24 hours period. During the later period at the time of starvation it falls gradually (Plate XIV, Graph E). Since M. pustulata dies or becomes inactive at the end of the third or fourth day following starvation no estimations could be made beyond this period. On the third day onwards nitrogen is not detectable with the present method used for estimation. During this period in the tubule tissue also very low quantity of nitrogen and protein is detected.

When the decrease in the amount of nitrogen and protein of tissue at 52 hours period is compared with the decrease in the concentration of phospholipid in the tubule of the same insect, it is found to be very little; but in the blood reduction in phospholipid is very significant after 48 hours period. At the same time immediately after feeding the previously starved insects both in the tubule and blood, the phospholipid concentration increases. In the case of experimental M. pustulata, nitrogen and protein in blood is found to be higher (8.475% and 52.96% respectively) in comparison to the same in the normal M. pustulata. But there is a higher level of phospholipid both in the tubule and blood (4.67% and 19.82% respectively) of experimental M. pustulata where as, the maximum levels of phospholipid concentration in the tubule and blood of the normal M. pustulata are 4.36 and 16.2% respectively.

L. maculatus shows a high level of total nitrogen and protein in blood immediately after feeding while in tubule it is delayed. Only at 16 hours period the nitrogen and protein in the tubule shows an increase (Plate XIV, Graph B and Plate XV, Graph F). At the time of 8 hours period the nitrogen content in the tubule homogenate is 0.007 mg/100 mg and total protein content is 0.043 mg/100 mg only. Though in blood and tissue the nitrogen and protein concentration increase gradually upto

48 hours, there is a low level point for the blood nitrogen and protein concentration at 16 hours period. After 48 hours period both in blood and tubule the total nitrogen and protein concentration falls gradually and becomes too low at 10 days period.

Phospholipid concentration of both tubule and blood increases gradually upto 48 hours, there is a low level point for the blood nitrogen and protein concentration at 16 hours period. After 48 hours period both in blood and tubules, the total nitrogen and protein concentration falls gradually, and becomes too low at 10 days period.

Phospholipid concentration of both tubule and blood increases in the L. maculatus after feeding. There is a negligible fall in the blood phospholipid level at 12 hours period when compared with the concentration at 8 hours period. Later it increases gradually upto the second day period, both in blood and tissue of the tubules and decreases gradually during starvation (Plate XIV, Graph G & H).

In L. maculatus it is found that the percentage of nitrogen, protein and phospholipids in the blood is higher than that in the tissue of the tubule. But in the case of M. pustulata during the third day of starvation no nitrogen or protein is detected in the blood.

Nitrogen and protein in the excreta:

Total nitrogen and protein in the excreta of normal feeding L. maculatus is 0.026-0.03 mg/100 mg and 0.16-0.18 mg/100 mg of excreta respectively, whereas, in the case of M. pustulata nitrogen and protein varies from 0.012-0.02 mg/100 mg and 0.075-0.12 mg/100 mg of excreta respectively (Plate XV, Graph A & B). Previously starved insects show a higher rate of nitrogen and protein excretion at 2 days period in the case of M. pustulata and at four days period in the case of L. maculatus. The rate of nitrogen excretion under normal condition is higher in L. maculatus than in M. pustulata. In the case of experimental M. pustulata since it is not possible to estimate the nitrogen after 48 hours no comparison is possible either with that of experimental or normal L. maculatus. Similarly, in the case of experimental M. pustulata excretion starts at 4 hours following feeding. It is calculated that the average nitrogen excretion in the experimental M. pustulata is 0.14%.

D I S C U S S I O N

Nitrogen:

There is a fair number of determinations on total nitrogen content of whole insect, blood, and excreta but that of a separate tissue or organ is meagre except for a few studies on the uric acid, an end product of nitrogenous metabolism in the fat body of Periplaneta americana (McEnroe and Forgash, 1957) inthe accessory sex glands of Blattella germanica (Roth and Dateo, 1964) and in the malpighian tubule of silkworm larva (Kuwana, 1987) and of P. americana (McEnroe, 1966). The effects of starvation on the composition of body nitrogen has been reported on Japanese beetle larvae (Newton, 1954) and on Tenebrio (Mellanby, 1932).

The data on the total nitrogen of whole insect varies with different workers. In Melolontha melolontha it is 3.28% of the fresh tissue (Dingler, 1927) while Slowtzoff (1909) has calculated it as 3.67% of fresh tissue. It comes 11.5% in Schistocerca paranesis (Kunckel d' Herculais, 1899), 12.08% in Palingenia laticauda (Zaitschek, 1904) of the dry substance and it is 10-20% in cane beetle, Lepiderma sp. (Jarvis, 1923). From these estimations it is evident that the average nitrogen

content of the insect is about 10% of the body weight and that the differences between species are not great.

In the case of M. pustulata and L. maculatus, the total nitrogen in the tubule homogenate shows variation according to the feeding conditions. The increase in the concentration of total nitrogen in the blood and tubules after feeding and decrease in the total nitrogen during the period of starvation in the blood and tubule are more or less parallel. The peak of nitrogen concentration reached in the blood after feeding in L. maculatus is reciprocal to that in tubules. As it declines during starvation it is evident that the total nitrogen in the blood and also in the malpighian tubule which is an organ through which the end products of nitrogenous metabolism are removed, depends on the feeding conditions. Recently, Ito and Fraenkel (1966) have shown that glucose fed larvae of Tenebrio contained higher proportion of ether extractable nitrogen than normal and starved ones.

Some preliminary observations on nitrogen gains are also recorded in the nymphs of Aeshna cyaneae (Staddon, 1959). The total nitrogen output prior to feeding ranges from 2.6-16.6 ug (ave:7 ugN)/100 mg wet weight/24 hours at 20°C and the feeding is followed by a bulk increase in nitrogen

output which lasted for 1-2 days. The total nitrogen output at the end of first day following feeding ranges from 17.7-46 ug (ave:32 ugN)/100 mg wet weight. The total nitrogen increases in the blood and tubules of M. pustulata immediately after feeding. The level increases gradually from 8 hours period to 24 hours period but in the L. maculatus the blood only show an increase in the concentration of total nitrogen from 8 hours period after feeding while in tubule it increases only from 12 hours period. This increase gradually continues in the blood and tubules of L. maculatus upto 48 hours period. But in the case of L. maculatus there is a low level point for the blood total nitrogen concentration at 16 hour period. In the case of M. pustulata at 24 hours period there is a sudden fall in the total nitrogen concentration of tubule whereas, the fall in the total nitrogen content of the blood is recorded as early as after 16 hours period. On the 4th day of starvation, M. pustulata either dies or becomes inactive while in the case of L. maculatus after 48 hours period, there is a gradual fall in the total nitrogen concentration in blood and tubules. L. maculatus can live in the starved condition even after 10th day.

The most possible reason for this difference between the two species of entirely different environment and feeding habits is provided by the food and the physiological process of

digestion and absorption. In the case of L. maculatus after a full meal the food ingested is stored in the crop and released slowly for digestion as it is the case with Rhodnius (Wigglesworth, 1931).

The concentration of tissue nitrogen in M. pustulata is always lower than the blood nitrogen except under starved condition. But in the case of L. maculatus the most interesting point is that even after continued starvation the nitrogen level in the tubule remains lower than that in the blood. Further in L. maculatus the total nitrogen in the tubule and blood is lower in comparison to the same in M. pustulata.

In the malpighian tubules the total nitrogen including the nitrogen present in the tissue plus the nitrogen produced as an end product of the metabolism is eliminated continuously from the tubule and thus the concentration of the tissue nitrogen in normal feeding insects is kept at a low level. During the starvation the blood volume becomes less due to the decrease in the volume of water which is an essential factor for the transport of nitrogenous end product from the tubule lumen to the gut. In Rhodnius (Wigglesworth, 1931) the volume of fluid excreted is proportionate to the total fluid ingested. Similarly, the rate of urine increases in

Schistocerca gregaria with increasing dilution of the haemolymph (Ramsay, 1953b).

In terrestrial insects the water lost during excretion is reabsorbed either by the rectum (Ramsay, 1952, 1955b; Philips, 1964; Berridge and Gupta, 1967; Wall and Oschmann, 1970; Hopkin et al., 1971; Hopkin and Srivastava, 1972) by crypto-nephric complex (Wigglesworth, 1934; Pradhan, 1942; Saini, 1964; Ramsay, 1964) or by proximal segment of malpighian tubule itself (Wigglesworth, 1931; Bahadur, 1964). During the excretory phase of D. fasciatus, osmoregulation is persumably achieved by drinking more liquid (Berridge, 1965) but in M. pustulata the chances of reabsorption of water through the crypto-nephric complex cannot be ruled out. However, under normal condition, M. pustulata being a continuously feeding insect and as its food contains 71.57% of water it probably gets enough water for the physiological routine. But even then the water is reabsorbed in M. pustulata through the well developed crypto-nephric complex. So it is reasonable to assume that the water reabsorbed during starvation period may be quite insufficient for the normal physiological activities. In the case of L. maculatus which is an aquatic insect the water may be ingested to balance the water loss during starvation. Since it feeds on liquid food and excretes liquid urine it may be concluded that the need to conserve

water is less urgent in L. maculatus than for any other terrestrial species. However, an alternative hypothesis that the higher concentration of tissue nitrogen in the tubules of M. pustulata during the starvation may be due to the endogenous origin of the products of nitrogen metabolism in the tubule cannot be ruled out as it has been suggested for the hypoxanthine of excreta which is not detected in the blood of Galleria mellonella (Nation and Patton, 1961). The haemolymph also stores a high amount of the end products of nitrogen is 1.34 gm/litre in the adult Dytiscus, 3.27 gm/litre in the pupa of Attacus, 3.2 gm/litre in the pupa of Sphinx and 2.34 gm/litre in the larva of Cossus (Duval et al., 1928). In the blood of Melolontha and Oryctes larva (Ussing, 1946) there is some 300-400 mg/litre of non protein amino nitrogen. The very high aminoacedemia with the stored end product of nitrogen may be one of the reasons for a high level of nitrogen in the blood than in the tissue. It is found that in L. maculatus which feeds on blood a nitrogenous material, show low concentration of nitrogen both in the tubule and blood in comparison to the blood and tubule of M. pustulata which feeds on plant matter. In the case of Aedes aegypti (Tarzian et al., 1957) when reared on different diets (Sucrose, blood, plasma) it is found that the total nitrogen remains constant. Though the mosquitoes maintained

on sucrose show a decrease in the percentage of tissue nitrogen during the week after emergence, but again during the following weeks there is a gradual increase in the tissue nitrogen. As it is reported by Auclair and Patton (1950) that the blood of milk weed bug contains a large amount of Dalanine which is not derived from the food but is the result of insect metabolism, it is reasonable not to expect a high concentration of total nitrogen in the case of L. maculatus because it feeds on a nitrogen rich food. It is also suggested that the decrease in the total nitrogen in the blood of M. pustulata may be due to utilization of stored nitrogen for metabolic processes during starvation. This will reduce the concentration of total nitrogen in the blood of M. pustulata. But in the case of L. maculatus as it is mentioned earlier, since food is digested periodically and absorbed in the blood, the nitrogen atleast in low concentration is found in the blood. It is also demonstrated in Rhodnius (Barret and Friend, 1966) that an increase in the rate of uric acid on the third day after feeding on artificial diet which contains no nitrogen is due to the remnants of previous blood meal in the gut. In the case of M. pustulata it is noticed that the gut after the 3rd day of feeding is completely empty except for the posterior half of the intestine.

Nitrogen in the excreta of L. maculatus is higher than that of M. pustulata. The proportion of the nitrogenous waste in the faeces of insect varies enormously depending to a large extent on feeding habits (Bursell, 1967). Since M. pustulata is a herbivorous species the nitrogen load in the excreta becomes light and the undigested plant material often makes up the bulk of faecal matter; but in the case of L. maculatus which is a blood sucking insect the nitrogen load is heavy in the excreta. The uric acid nitrogen in herbivorous insect comprises not more than 0.1% of dry weight (Razet, 1961) while it may account for as much as 60% of dry weight of faeces in blood sucking insects (Bursell, 1964). In the case of Aeshna cyanea, Staddon (1959) has reported that the nymphs excrete within 24-48 hours after feeding, a quantity of nitrogen equivalent in amount to 60% or more of the total nitrogen absorbed during that period. The excreta of Sialis lutaria (Staddon, 1955) contains total nitrogen of 11.0 ug/100 mg wet weight/24 hours. Terzian et al. (1957) have shown that on a diet of sugar the proportion of uric acid nitrogen in the excreta falls to about 4% but following a blood meal the nitrogen output increases greatly. Faecal analyses for the nitrogenous compounds of boll weevils Anthronomus grandis (Mitlin et al., 1964) show that the total nitrogen differs greatly with the type of diet. The faeces

derived from the square fed weevils contains nitrogen far in excess of those derived from boll fed or artificially fed weevils. In square fed weevils the total nitrogen is 45.42 mg/g weight of faeces while in the boll fed and artificially fed weevils the excreta contains 33.41 mg/g and 29.96 mg/g weight of the faeces. Diet containing from 5% to 30% protein produced no major change in either total purine nitrogen excreted or purine nitrogen partition of the larva of wax moth while higher percentage of protein in the diet increase the amount of purine nitrogen excreted and shift the purine nitrogen partition towards increased excretion of hypoxanthine and xanthine (Nation and Thomas, 1965). The pure protein diet involves the excretion of ammonia and uric acid about twice as great as the normal meat diet in certain muscid flies (Brown, 1936). In the case of Aeshna cyanea (Staddon, 1955) when fasting nymphs are fed on a protein diet in the form of egg white there is a large temporary increase in the end products of nitrogen metabolism.

From the data it is observed that in the normal feeding insects, a consistancy in the concentration of nitrogen both in the blood and tissue of L. maculatus and M. pustulata is maintained. The studies on silk worm (Inoye, 1912) and on Malacasoma americana (Russo, 1922; Rudolfs, 1926) show that on the whole the nitrogenous substances are of importance only at critical stages of development. As far as the adult is concerned for the developments of eggs, nitrogen is essential

(Baumberger, 1919; Nelson et al., 1924; Snodgrass, 1925; Parker, 1926, Bertholf, 1927). Since only males were used in the present study no information on nitrogen fluctuations if any in the females can be provided.

Protein:

The determination made on the protein percentage in insects, for instance in humble bee it is 18.11% (Slowtzoff, 1905) and in Melolontha melolontha it is 17.13% (Slowtzoff, 1909) of fresh weight, showing that it varies greatly from one insect to other. Studies on adults have revealed that protein concentration in the blood can be greatly influenced by nutrition (Chen, 1966). The observation of present author also shows that the concentration of protein in the tissue is also influenced by nutrition. During the period of starvation there is a great decrease in the concentration of protein both in the blood and tissue of M. pustulata and L. maculatus. Previously starved ones show a gradual increase in the protein concentration. The increased rate of protein in the blood requires an enhanced rate of protein digestion. In the case of Musca domestica, total haemolymph protein increases in the carbohydrate fed males and females; substancial increase in the haemolymph protein occurred after one day of milk feeding indicates that dietary protein is made available as haemolymph protein relatively quickly (Bodnaryk and Morrison,

1966). M. pustulata and L. maculatus, when continuously fed show no accumulation of protein either in the tubule or in the blood. It is probably due to the lesser requirements of reserve material by the testes for the growth and development because haemolymph protein in the females has been shown to be significantly higher than males fed on the same diet (Martgnoni and Milstead, 1964). The present observation that protein level is more or less constant in the blood and tubule when the insect is regularly fed lends support to the finding of Van der Geest (1968) that there is no significant difference between the maximum concentration of total protein in the fifth instar larva when continuously fed and reared on different diets.

Blood proteins are not detected by Tobe and Loughton (1967) in tissue of Locusta migratoria migratorioides at any stage of development with the exception of ovary of the adults. Similarly, in Malacosoma americana neither abdominal tissue nor malpighian tubule show any sign of haemolymph protein at any time during the life cycle (Loughton and West, 1965). But the sequestration and storage of haemolymph protein by fat body cells during the larval pupal transformation has been demonstrated in Calipodes ethlius (Locke and Collins, 1968), and in Pieris brassicae (Chippendale and Kilby, 1969). But Locke and Collin (1968) and Tobe and

Loughton (1969) have shown that in Locusta during the last nymphal instar labelled haemolymph proteins are taken by the fat body and other tissues. An explanation for the increase in the protein in the level of tissue after feeding is provided by extending the hypothesis of Siakotos (1960 a,b) which suggests blood protein as a carrier of nutrients such as lipids and carbohydrates. It may be possible that proteins are absorbed for the conjugated groups. The proteins would then enter the cell as a side group joined to the essential nutrient. That protein and conjugated group do enter the cell is demonstrated by incorporation of antigen 5 and its blue green chromatophore into the heart muscle of M. americana (Loughton and West, 1965). The fat body extracts of Galleria mellonella contain glycoprotein during both larval and pupal stages and it is taken for granted that these would be sequestered from the haemolymph. After electrophoresis these bands of the spinning stage fat body stained for glycoprotein supporting the conclusion that these bands represent protein sequestered from haemolymph (Collins and Downe, 1970). In the case of M. pustulata and L. maculatus it is found that muco- or glyco-proteins are found in the tubule and it is observed to vary in the concentration according to the feeding and starvation. Butterworth et al., (1965) suggest that in Drosophila the storage granules of fat body stained with PAS method

contain glycoproteins.

Many enzymes which are essential for metabolism are present in the malpighian tubule (Mazzi and Baccetti, 1956; 1957a; Kilby and Neville, 1957; Auclair, 1959; Berridge, 1965). During the fed condition the tubule tissue is active and the enzymes are produced for the normal functioning. This may also be one of the reasons for higher concentration of protein in the tubule during fed condition. Similarly, during the starvation period due to low level metabolism in the tubule tissue the enzymes may be present in low quantity or even absent. So the level of protein becomes less during starvation.

Proteins are used extensively during starvation in Dytiscus (Pilewiczowna, 1926). In adult Celerio it utilises 41% of its protein during starvation (Heller, 1926) before death. It has been estimated that 22% of the protein is used in Melolontha and 20% of protein in Geotrupes during starvation (Slowtzoff, 1904, 1905). In starved mosquito larval protein in all the tissues especially that of fat body and muscles is used (Wigglesworth, 1942). In the case of Odonata the protein content falls down 53.4-51.7% of the body weight while in Bombus it falls from 58.3% - 52.1% (Slowtzoff, 1904, 1905, 1909). But in Apis it is showed that atleast at 23°C

there appears to be no utilization of protein at all during fasting (Keller Kitzinger, 1935). Starvation has been demonstrated to bring about significant reduction in the total protein (Beadle and Shaw, 1950). These evidences give firm support to the present observation that there is a decrease in the concentration of protein in the blood and malpighian tubule tissue of both M. pustulata and L. maculatus during starvations. It has been clearly shown in the blowfly, Phormia by recent work of Orr (1964a) during forced starvation the blood protein drops to extreme low levels. Since parallel observation is found in the tissue it can be persumed that the tissue protein may serve as an important reserve as it is the case with haemolymph protein (Chen, 1966). In Popillia larva body protein is used as an energy source during starvation (Newton, 1954). During this period the unfed insect consumes its reserves for the production of energy. In the case of larvae of Aedes swimming in clear water at 28°C, protein in all tissues are greatly reduced in 10-12 days (Wigglesworth, 1942). Though no significant reduction in the absolute protein content occurred during one month of diapause in the Trogoderma granarium, the absolute protein content after 9 months starvation falls from 103.9 mg/100 - 45.45 mg/100 larva (Karnavar and Nair, 1969). The low concentration of protein in the excreta of M. pustulata and

and the higher level of protein in the excreta of L. maculatus may be due to the difference in the feeding habits as it is the case with nitrogen.

Phospholipids:

Both in the case of M. pustulata and L. maculatus though there is a fall in the concentration of phospholipid in the tubule at the time of starvation it is not significant as it is the case with nitrogen and protein. But the loss in the case of blood is more significant, going down in M. pustulata from 19.75 mg/100 ml to 10.9 mg/100 ml at 48 hours period during starvation. Similarly, in L. maculatus the phospholipid level decreases from 15.6 mg/100 ml to 4.25 mg/100 ml. Though informations on the tissue lipids of other insects are not available except for silk worm (Shridhara and Bhat, 1965), it is worth mentioning that the phospholipid concentration in the continuously feeding M. pustulata and L. maculatus show very little difference either in the tubule or blood. A comparative account of phospholipid in different tissues has been given by Sridhara and Bhat (1965). The total phospholipid content is 0.2-0.4% in sugar beetle web worm (Pepper and Hastings, 1943), 3.5% in Calliphora, 2.0% in Tenebrio, 3.0% in Schistocerca, 3.0% in (Albrecht, 1961). The study of phospholipid in the subcellular

fractions of housefly shows that mitochondria contain 28.1% and microsome contain 23.4% of total phospholipid (Khan and Hodgson, 1967). Ludwig (1950) has shown that the loss in lipid nitrogen is much greater than that of soluble nitrogen during starvation in the grasshopper Chortaphaga viridifasciata. An increase in phospholipid immediately following feeding in the previously starved insect has been observed in the blood and tubule of both M. pustulata and L. maculatus. Similarly, the lipid nitrogen is greatly increased in the glucose fed larva of T. molitor. This fraction consisting of phospholipids is involved in the mobilization and transport of fat (Ito and Fraenkel, 1966).

Since most of the studies are on the phospholipid of whole insects it is difficult to collaborate the present finding with that of earlier works due to the fact that the composition of individual organs may be quite different from that of whole insect. Mitochondria of housefly contain more phospholipid than in microsomes (Khan and Hodgson, 1967). Similarly, they suggest that in other tissues the phospholipid content varies from one membrane to the other. Crone (1964) finds that the flight muscle's sarcosomes of housefly contain more phosphatidylethanolamine and less phosphatidylcholine than the intact flies. Allen and Newburgh (1965) have reported

the presence of lysophosphatidylcholine in the fat body of Sarcophaga bullata.

From the studies on the phospholipid concentration of malpighian tubules of both M. pustulata and L. maculatus it seems reasonable to assume that phospholipid also plays an important role in the metabolic activities of the tubule as it is the case with nitrogen and protein. Studies on the phospholipid during the development (Bieber et al., 1961; Bridges and Cox, 1962; Sridhara and Bhat, 1965; D'costa and Birt, 1966; Weimer and Lumb, 1967) show that there is a decline in the phospholipid concentration at the period of larval and pupal transformation. D'costa and Birt (1966) have gone a step further and assumed that relative consistancy during metamorphosis may be due to a balance of degradation and synthesis as probably occurs for the nitrogen compounds. Since phospholipid is presumably involved in protein synthesis and secretion (Hokin and Hokin, 1956; Hendler, 1958) and as protein synthesis increases just prior to pupation, Weimer and Lumb (1967) suggest that the change in the distribution of fatty acids and phospholipids is related to an increase in protein synthesis. The study of Thomas and Gilbert (1967) on Hyalophora cecropia and Periplaneta americana has proved that the insect fat body has the capacity to

synthesise phospholipids from simple precursors and release this phospholipid into the haemolymph from where it is transported in the form of lipoprotein.

Nevertheless, more significant point is that the concentration of phospholipid in the malpighian tubule is higher than that of nitrogen in both M. pustulata and L. maculatus. There is no doubt that phospholipid plays a variety of role in the life of cell. Their presence in the cell membrane has been unequivocally accepted viz., the human erythrocyte membrane contains about 59% phospholipid (Wolfe, 1964) where as, the RNA free microsomal fraction of neuronal membrane contains about 65% phosphatides. Phospholipids are also important component of membranes of cell organelles and the spatial configuration of the mitochondria depends on the presence of requisite phospholipids (Petrushka et al., 1959). From a figure quoted by Fleischer et al (1962) for heavy mitochondria of beaf heart a value of 325 mg phospholipid per gram of protein is derived and a value of 193 mg/g can be obtained (Getz et al. (1962) for rat liver mitochondria.

The present observation regarding higher phospholipid composition of malpighian tubule may be explained taking into consideration its role in the oxidative phosphorylation (Van Den Bergh and Slater, 1962; Green and Fleischer, 1963) and

in membrane transport (Sacktor and Dick, 1962). The role of mitochondria in the cellular physiology of malpighian tubule is well known (Beams et al., 1955; Meyer, 1957; Berkaloff, 1959 and Wigglesworth and Salpeter, 1962). Without phospholipids the mitochondria cease to function. In addition phospholipid may be an energy source for the cell since fatty acid moietus of the molecules can be oxidised to yeild relatively high quantity of energy in the form of ATP (Gilbert, 1967). The study of Ramsay (1953 a,b, 1955 b) proves that water, potassium and sodium are actively transported into the malpighian tubules of insects against a chemical gradient which requires the expenditure of energy. The presence of glycolytic-kreb cycle pathway in the tubule and the susceptibility of tubule to low dosages of azide, cyanide, and dinitrophenol indicates that energy formation is geared to oxidative phosphorylation (Berridge, 1968). Hence it is reasonable to put forth the view that the strategically arranged mitochondria which manufacture and make available the necessary supply of ATP (Berridge and Gupta, 1967, 1968) are responsible for higher level of phospholipids in malpighian tubules.

R E F E R E N C E S

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PLATE I

Fig. 1. Displacement of malpighian tubules in *C. indicus*.

Fig. 2. A portion of tubule showing muscular strand im *C. indicus*.

Fig. 3. The junction between the ampulla and the gut in *C. indicus*.

Fig. 4 & 5. T.S. of the first region of malpighian tubule showing various types of cells in *C. indicus*.

Fig. 6. L.S. of the second region of malpighian tubule of *C. indicus*.

Fig. 7. T.S. of the third region of malpighian tubule of *C. indicus*.

Fig. 8. T.S. of the fourth region of malpighian tubule of *C. indicus*.

Fig. 9. L.S. of the ampulla showing the opening of the tubule in *C. indicus*.

Amp. ampulla; BMb. basement membrane; C_1. first type of cell; C_2. second type of cell; C_3. third type of cell; C_4. fourth type of cell; FcMb. infoldings of the cell membrane; GR. granules; HG. hindgut; Ic. interstitial cell; Lum. lumen; Mal. malpighian tubule; mcl. muscle layer; MG. midgut; N. nucleus; Nl. nucleolus; :. Pl. peritoneal layer; sb. striated border; Tra. tracheole; Z_1. basal zone; Z_2. central zone; Z_3. border zone.

PLATE I

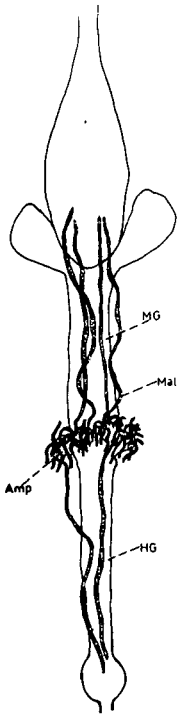
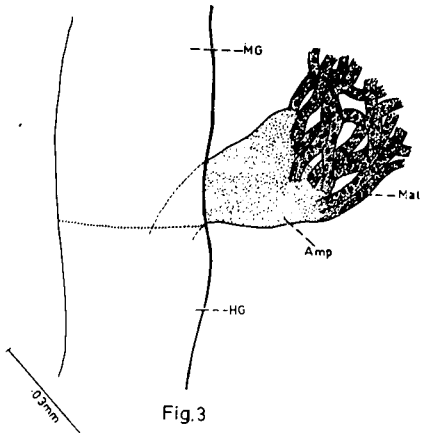


Fig.1



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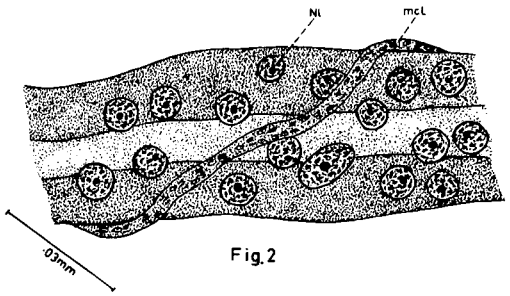

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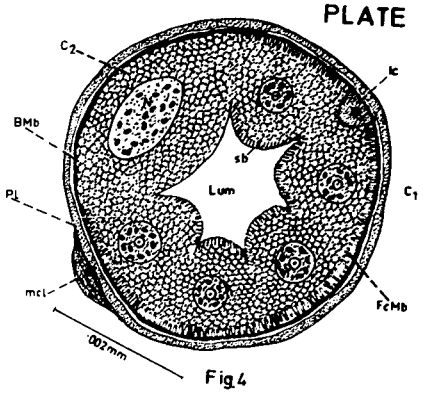

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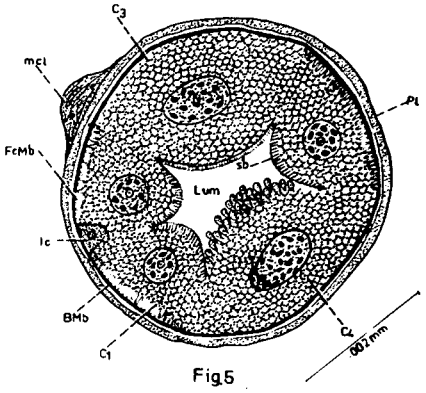

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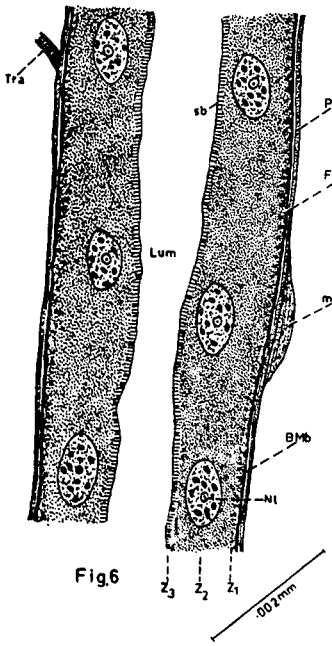

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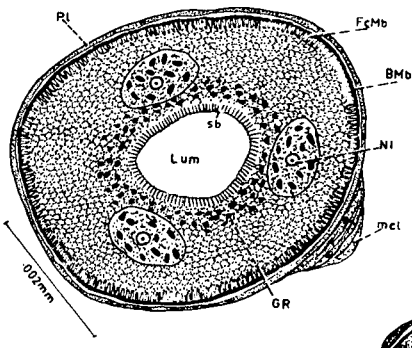
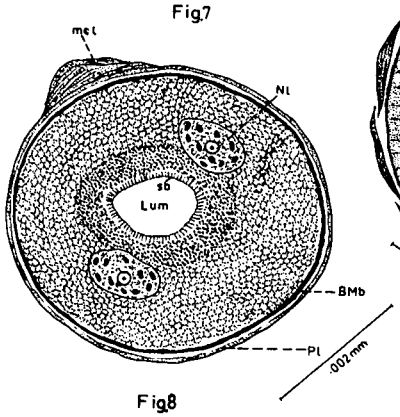


Fig.7



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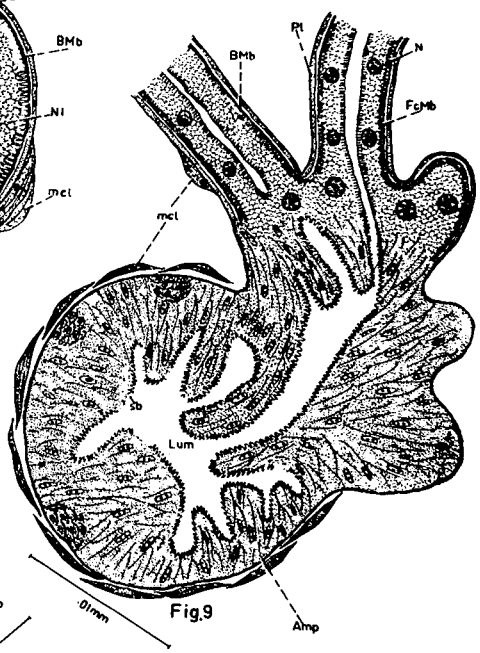


Fig.9

PLATE II

Fig. 10. L.S. showing the ampulla opening into the gut lumen in C. indicus.

Fig. 11. Displacement of malpighian tubules in S. praciniferum.

Fig. 12. The junction between the ampulla and gut in S. praciniferum.

Fig. 13. A portion of the tubule showing the muscular strand in S. praciniferum.

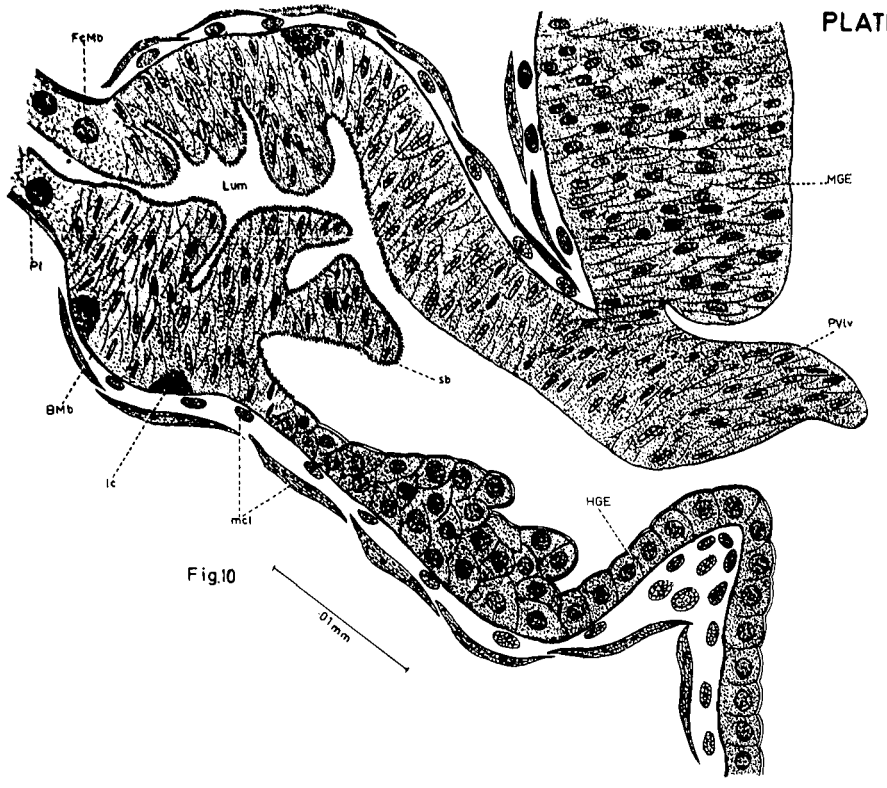
Fig. 14. T.S. of the first region of the malpighian tubule of S. praciniferum.

Fig. 15. T.S. of the third region of the malpighian tubule of S. praciniferum.

Fig. 16. L.S. of the second region of the malpighian tubule of S. praciniferum.

Amp. ampulla; BMb. basement membrane; FcMb. infoldings of the cell membrane; GR. granules; HG. hindgut; HGE. hindgut epithelium; Ic. interstitial cell; Lum. lumen; Mal. malpighian tubule; mcl. muscle layer; MG. midgut; MGE. midgut epithelium; N. nucleus; Nl. nucleolus; Pl. peritoneal layer; PVlv. ventricular valve; sb. striated border; V. vacuole.

PLATE II



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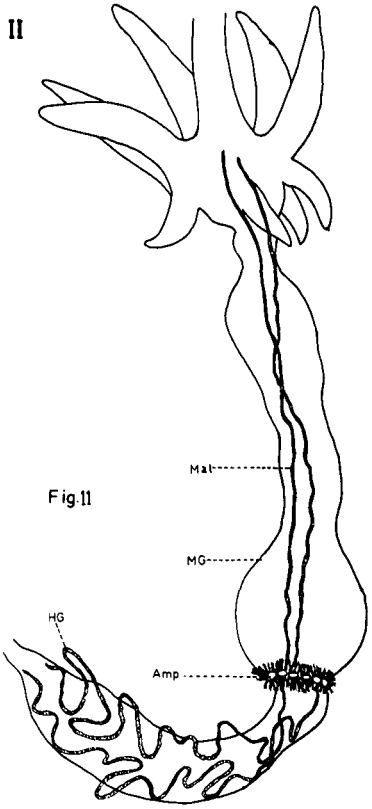

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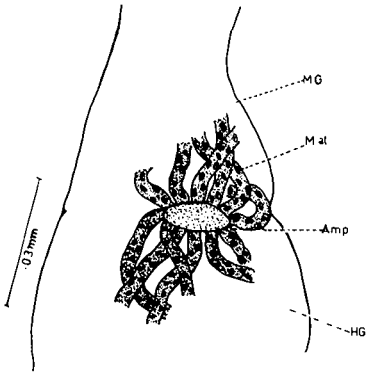

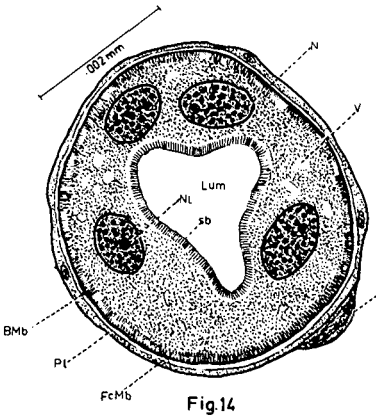

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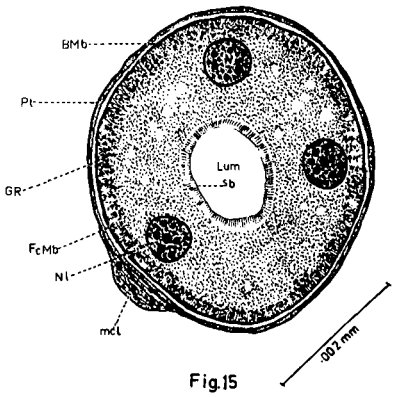

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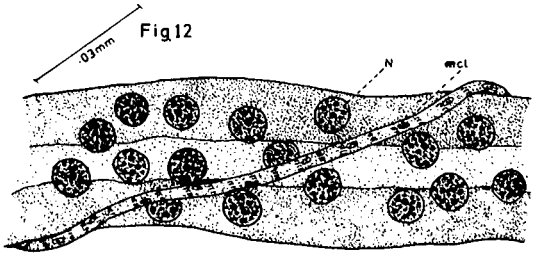


Fig.12

Fig.13

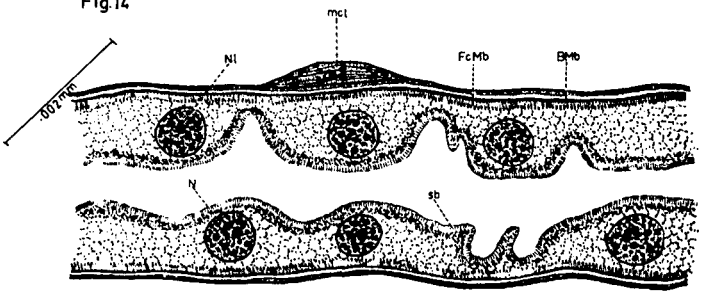


Fig.16

PLATE III

Fig. 17. L.S. showing the continuity between the muscle layer of the tubule and gut wall in S. praciniferum.

Fig. 18. Horizontal section showing the opening of the ampulla into the lumen of the tubule in S. praciniferum.

Fig. 19. T.S. showing the fusion of muscle fibers of neighbouring tubules in S. praciniferum.

Fig. 20. L.S. showing the opening of the ampulla into the gut lumen of S. praciniferum.

Fig. 21. Displacement of malpighian tubules in L. maculatus.

Amp. ampulla; BMb. basement membrane; Cch. common chamber; FcMb. infoldings of the cell membrane; FGE. foregut epithelium; HG. hindgut; HGE. hindgut epithelium; Lum. lumen; mcl. muscle layer; N. nucleus; Nl. nucleolus; Pl. peritoneal layer; PVlv. ventricular valve; Py. pylorus; Rect. rectum; Reg_1. first region of the malpighian tubule; Reg_2. second region of the malpighian tubule; Reg_3. third region of the malpighian tubule; Reg_4. fourth region of the malpighian tubule; sb. striated border; Tra. tracheole.

PLATE III

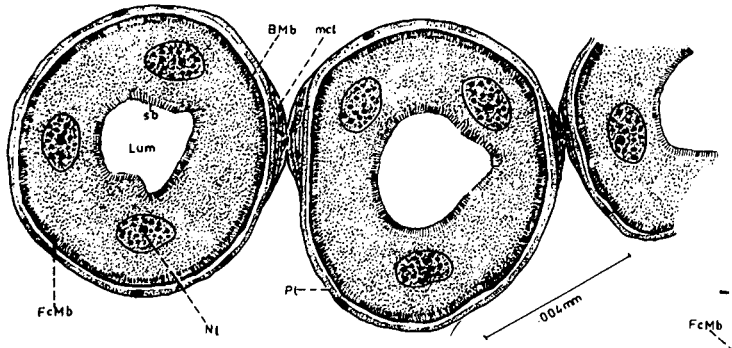
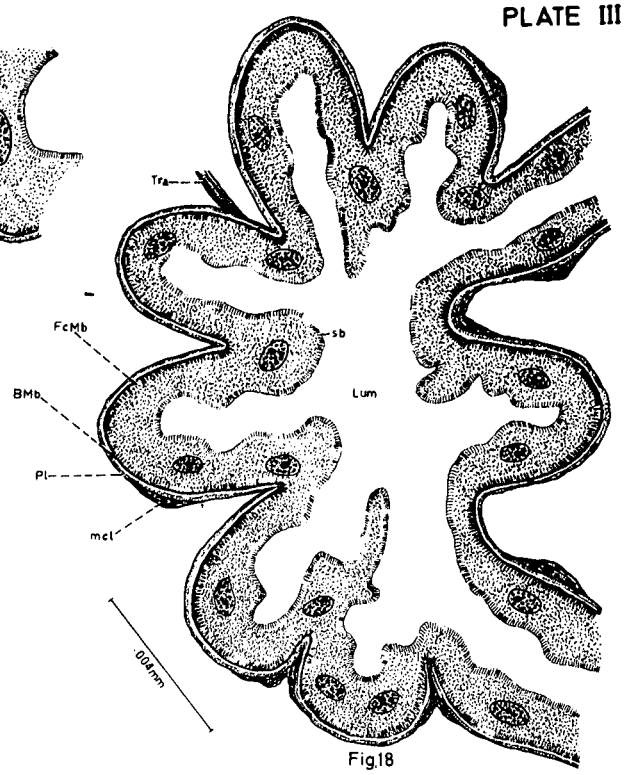


Fig.19



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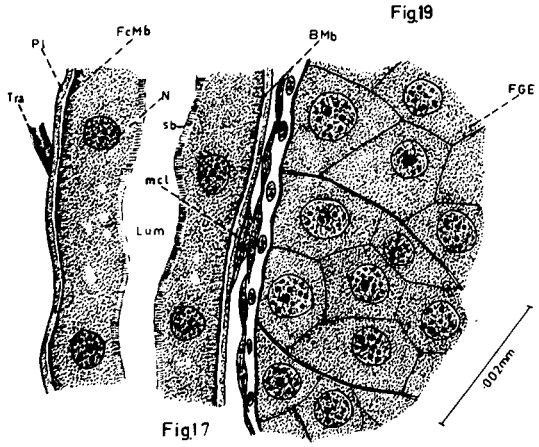


Fig.17

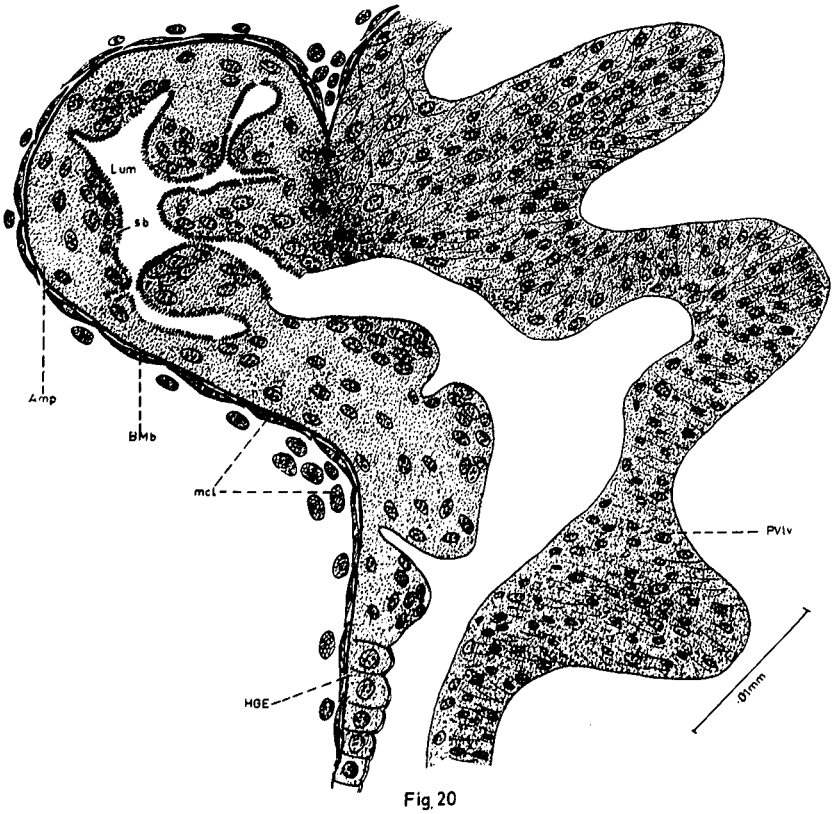


Fig. 20

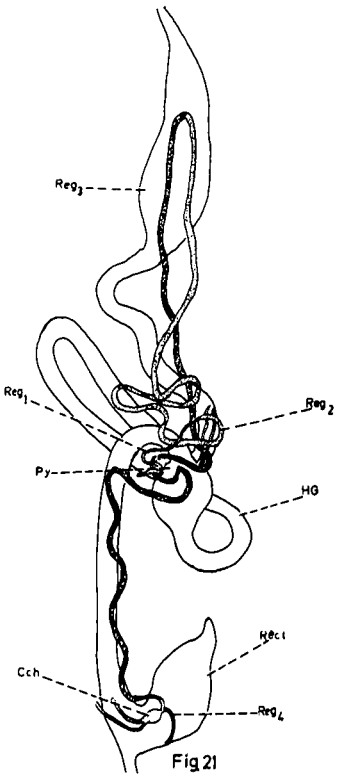


Fig.21

PLATE IV

Fig. 22. The junction between the tubule and gut in *L. maculatus*.

Fig. 23. L.S. of the proximal part of the first region of malpighian tubule of *L. maculatus*.

Fig. 24. L.S. of the distal part of the first region of malpighian tubule of *L. maculatus*.

Fig. 25. L.S. of the second region of the malpighian tubule of *L. maculatus*.

Fig. 26. L.S. of the third region of the malpighian tubule of *L. maculatus*.

Fig. 27. L.S. of the fourth region of the malpighian tubule of *L. maculatus*.

Fig. 28a. T.S. showing the malpighian tubules in the gut wall lying between the epithelium and muscle layer in *L. maculatus*.

Fig. 28b. T.S. showing the common duct of the tubules in the gut wall of *L. maculatus*.

Fig. 28c. T.S. showing the common duct opening into the gut lumen in *L. maculatus*.

BMb. basement membrane; Ct. connective tissue; FcMb. infoldings of the cell membrane; gLum. lumen of the gut; Lum. lumen; GR. granules; Mal. malpighian tubules; mcl. muscle layer; MG. midgut; MGE. midgut epithelium; N. nucleus; Nl. nucleolus; Pl. peritoneal layer; Py. pylorus; sb. striated border.

PLATE IV

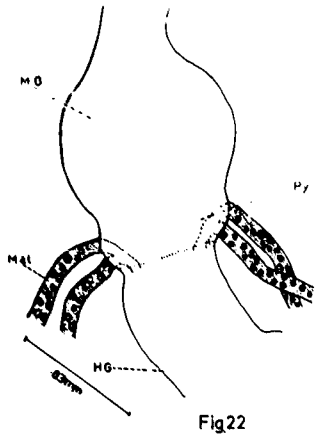


Fig.22

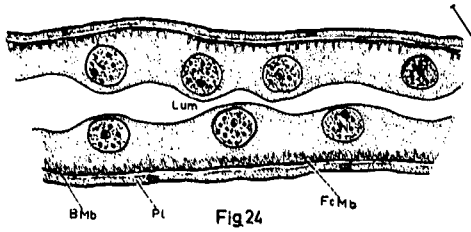


Fig.24

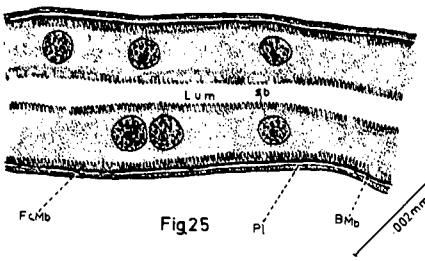


Fig.25

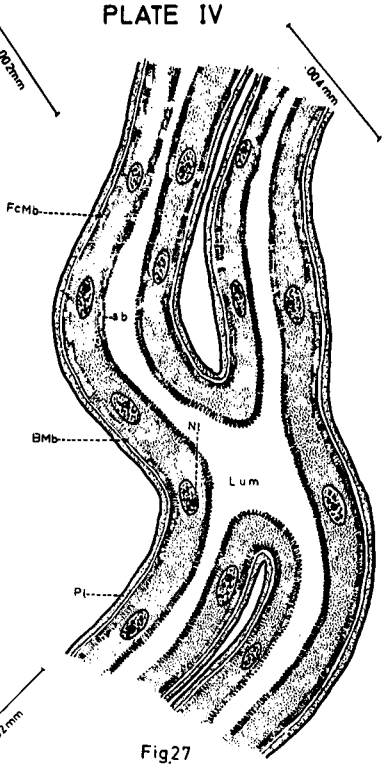


Fig.27

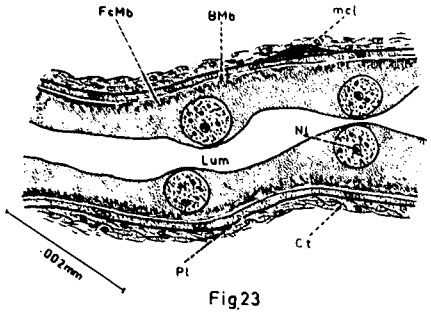


Fig.23

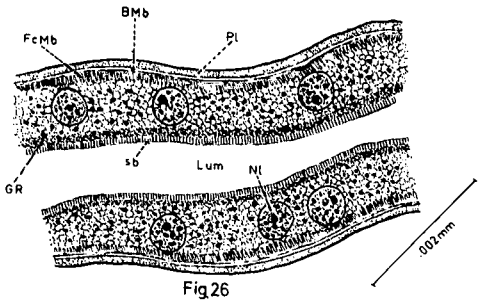


Fig.26

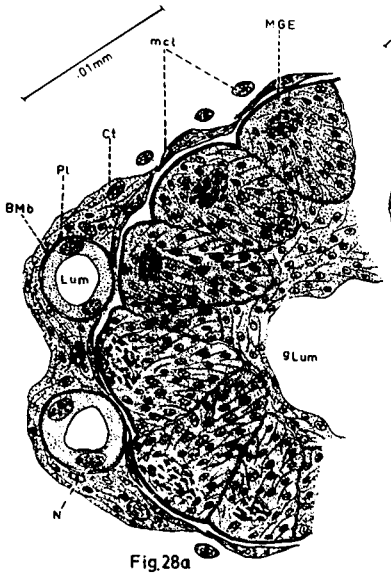


Fig.28a

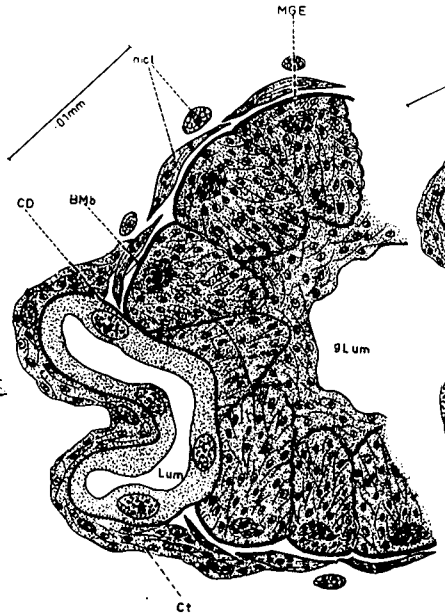


Fig.28b

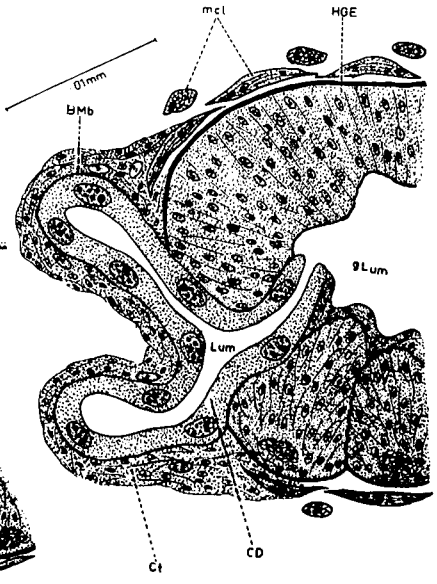


Fig.28c

PLATE V

Fig. 29. Displacement of the tubules in *M. pustulata*.

Fig. 30. The junction between the tubule and the gut in *M. pustulata*.

Fig. 31. T.S. of the first region of the free tubule in *M. pustulata*.

Fig. 32. T.S. of the second region of the free tubule in *M. pustulata*.

Fig. 33. T.S. of the third region of the free tubule in *M. pustulata*.

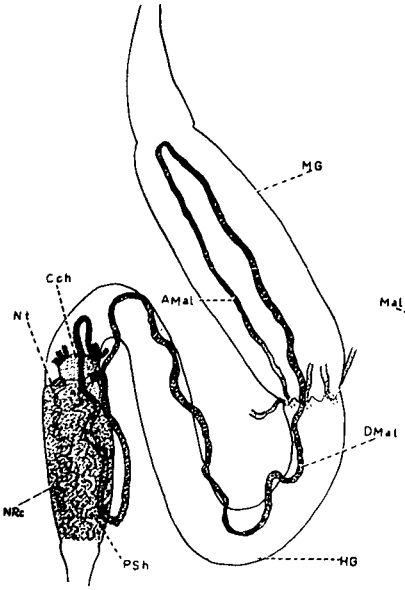
Fig. 34. Horizontal section of the common chamber formed by the fusion of free tubules in *M. pustulata*.

Fig. 35. L.S. of the first region of the cryptonephric complex in *M. pustulata*.

Fig. 36. L.S. of the second region of cryptonephric complex in *M. pustulata*.

AMal. ascending limb of free tubule; BMb. basement membrane; Cch. common chamber; DMal. descending limb of free tubule; FcMb. infoldings of the cell membrane; HG. hindgut; HGE. hindgut epithelium; Lph. leptophragma; Lum. lumen; mcl. muscle layer; MG. midgut; N. nucleus; NRc. cryptonephric complex; Nt. nephric tubule; PE. pronephric epithelium; Pl. peritoneal layer; PMb. pronephric membrane; Ps. pronephric space; Psh. pronephric sheath; sb. striated border

PLATE V



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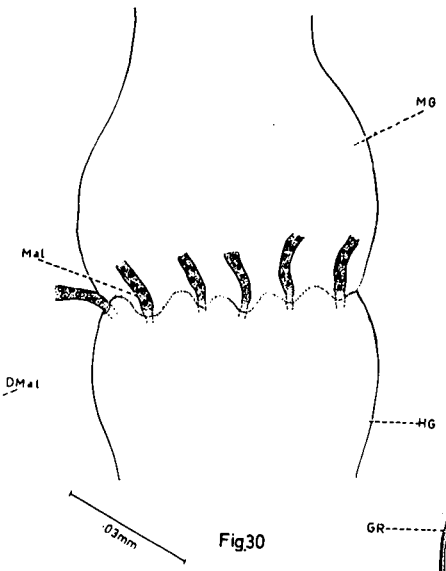
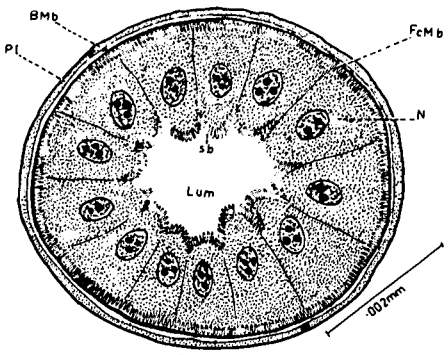


Fig.30



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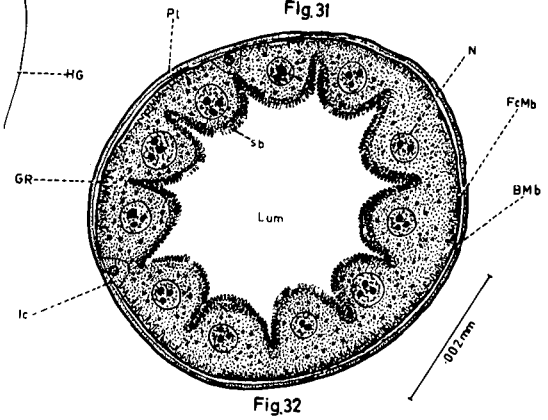


Fig.32

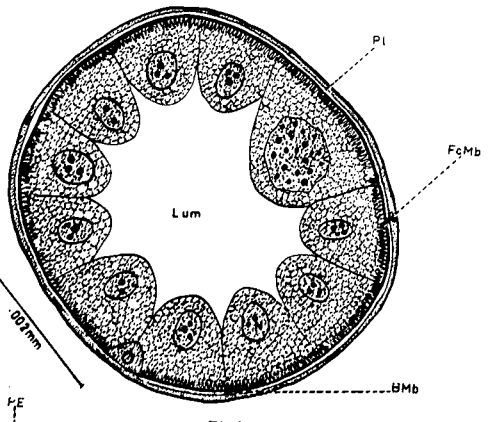
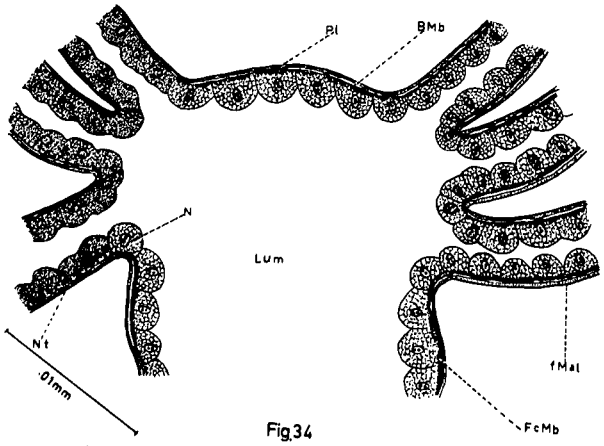


Fig.33



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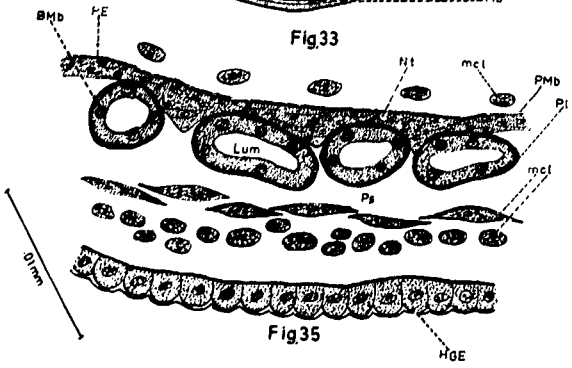

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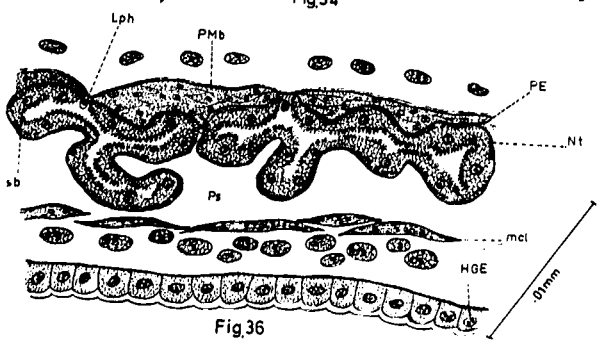


Fig.36

PLATE VI

Fig. 37. L.S. of the third region of crypto-nephric complex in *M. pustulata*.

Fig. 38. A portion of the crypto-nephric complex showing the arrangement of nephric tubules in the first and second regions of the complex in *M. pustulata*.

Fig. 39. A portion of the crypto-nephric complex showing the arrangement of nephric tubules in the third region of the complex in *M. pustulata*.

Fig. 40. A portion of the crypto-nephric complex showing the arrangement of nephric tubule in the fourth region of the complex in *M. pustulata*.

Fig. 41. L.S. showing the different structures involved in crypto-nephric complex in *M. pustulata*.

Fig. 42. T.S. showing type 1 leptophragma in *M. pustulata*.

Fig. 43. T.S. showing type II leptophragma in *M. pustulata*.

Fig. 44. T.S. showing type III leptophragma in *M. pustulata*.

F

BMb. basement membrane; FcMb. infoldings of the cell membrane; HGE. hindgut epithelium; In. intima; Lum. lumen; Lph. leptophragma; mcl. muscle layer; N. nucleus; Nb. nephric bud; Nt. nephric tubule; PE. pronephric epithelium; PMb. Pronephric membrane; sb. striated border; sEHG. syncytial epithelium of the hindgut.

PLATE VI

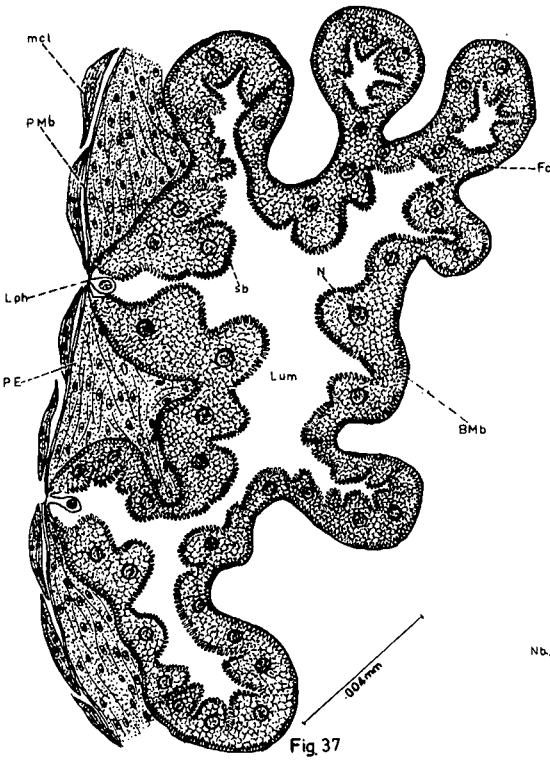


Fig. 37

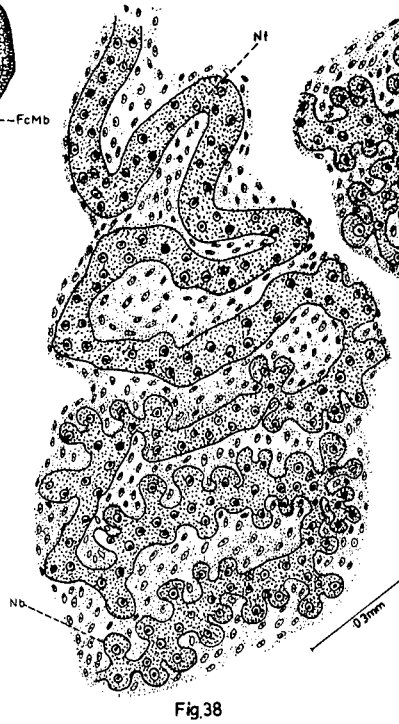
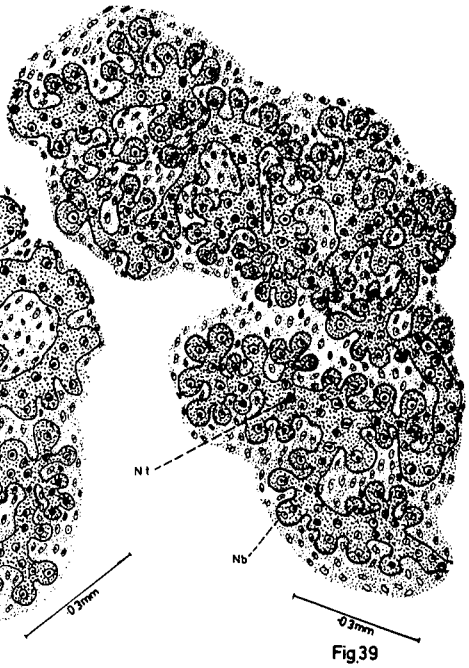


Fig.38



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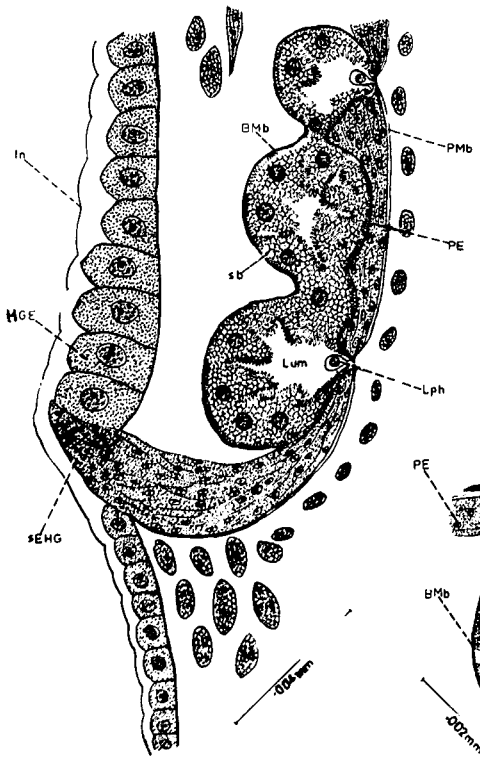

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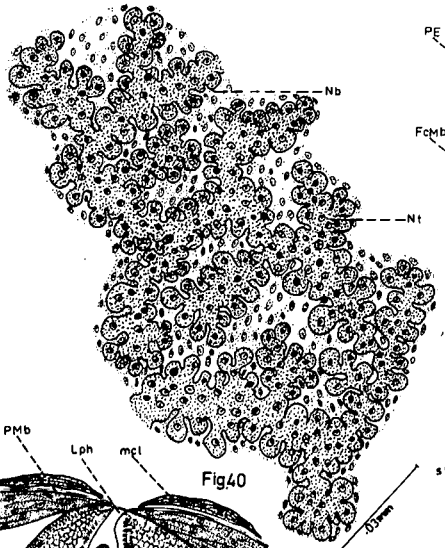


Fig.40

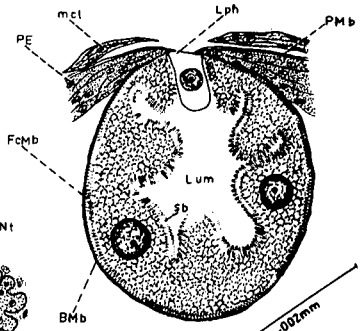


Fig.43

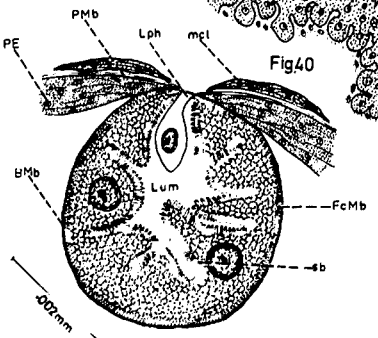


Fig.42

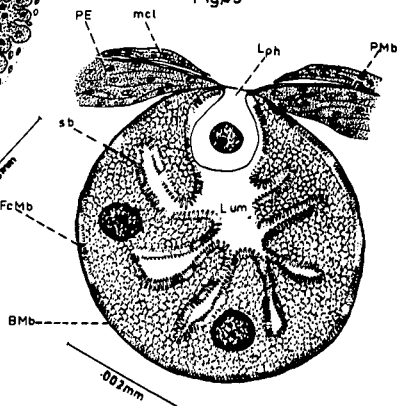


Fig.44

PLATE VII

Fig. 45. L.S. showing the malpighian tubule opening into the gut lumen in *M. pustulata*.

Fig. 46. Displacement of the malpighian tubules in *C. septumpunctata*.

Fig. 47. The junction between the malpighian tubules and the gut in *C. septumpunctata*.

Fig. 48. L.S. of the first region of the malpighian tubule of *C. septumpunctata*.

Fig. 49. T.S. of the second region of the malpighian tubule of *C. septumpunctata*.

Fig. 50. T.S. of the third region of the malpighian tubule in *C. septumpunctata*.

Fig. 51. T.S. of the fourth region of the malpighian tubule in *C. septumpunctata*.

Fig. 52. A portion of the crypto-nephric complex showing the arrangement of nephric tubule in the first region of complex in *C. septumpunctata*.

BMb. basement membrane; FcMb. infoldings of the cell membrane; GR. granules; HG. hindgut; HGE. hindgut epithelium; In. intima; Lum. lumen; Mal. malpighian tubule; Mcl. muscle layer; MG. midgut; MGE. midgut epithelium; N. nucleus; Nl. nucleolus; NRc. crypto-nephric complex; Nt. nephric tubule; PE. pronephric epithelium; Pl. peritoneal layer; ProcVlv. proctodaeal valve; Reg_1. first region of the free tubule; Reg_2. second region of the free tubule; Reg_3. third region of the free tubule; sb. striated border; st. short tubule.

PLATE VII

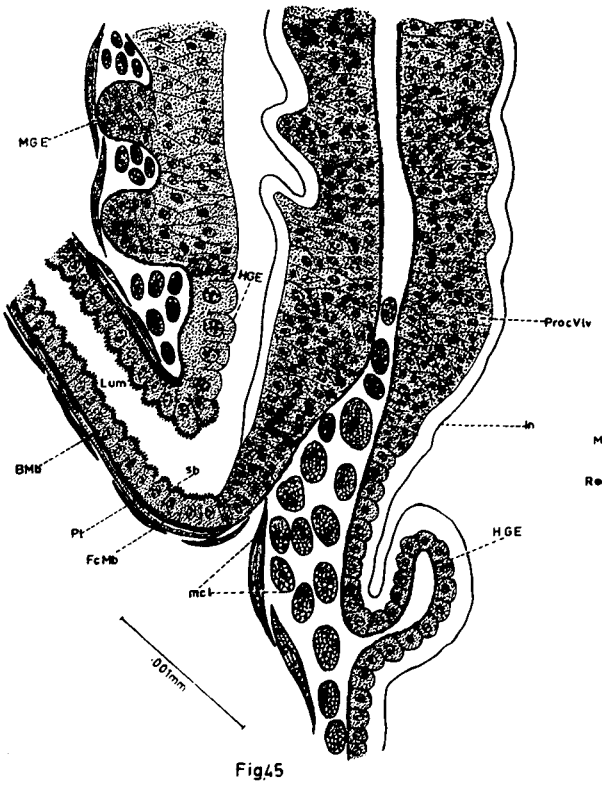
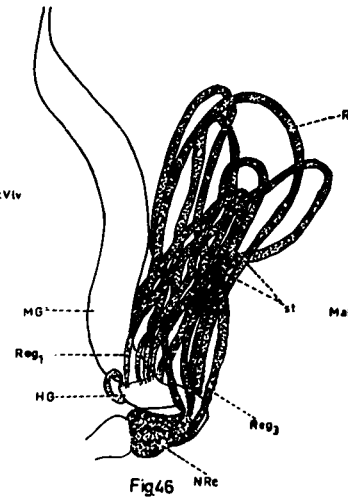


Fig.45



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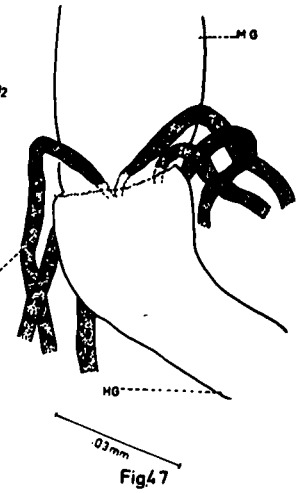


Fig.47

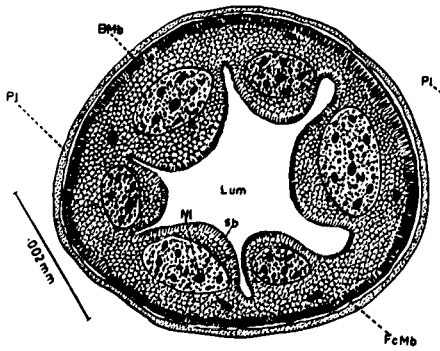


Fig.51

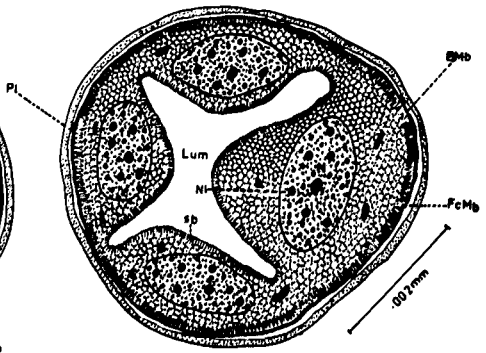


Fig.50

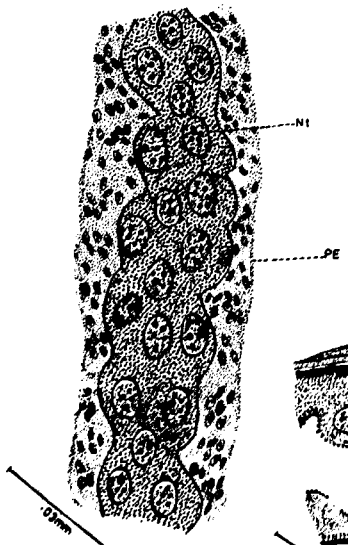


Fig.52

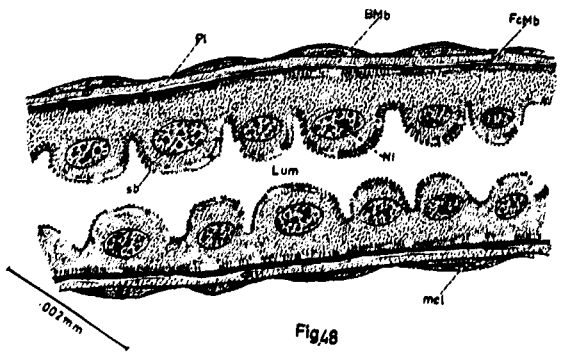


Fig.48

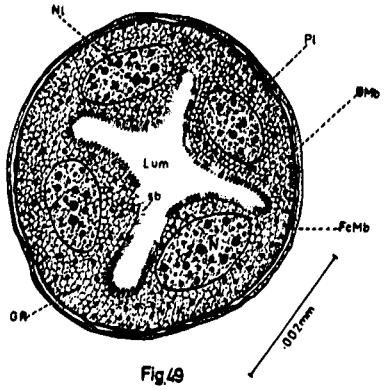


Fig.49

PLATE VIII

Fig. 53. A portion of the crypto-nephric complex showing the arrangement of nephric tubules in the second region of the complex in C. septumpunctata.

Fig. 54. A portion of the crypto-nephric complex showing the arrangement of nephric tubules in the third region of the complex in C. septumpunctata.

Fig. 55. T.S. showing the leptophragma in C. septumpunctata.

Fig. 56. T.S. showing the leptophragma with the pronephric membrane pushed towards the outer side in C. septumpunctata.

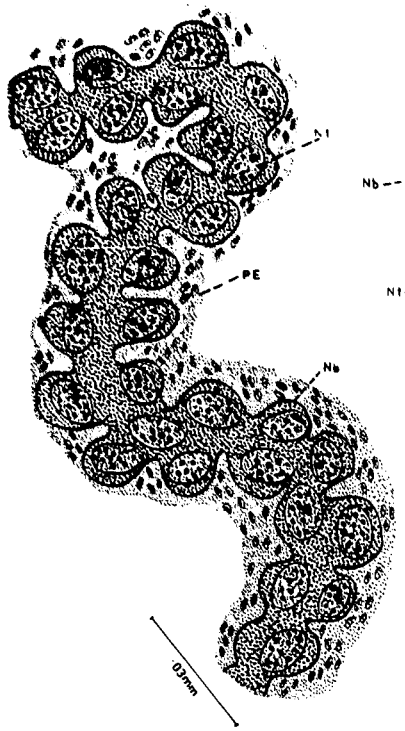
Fig. 57. T.S. of the first region of crypto-nephric complex in C. septumpunctata.

Fig. 58. T.S. of the second region of crypto-nephric complex in C. septumpunctata.

Fig. 59. T.S. of the third region of crypto-nephric complex in C. septumpunctata.

BMb. basement membrane; HGE. hindgut epithelium; Lph. leptophragma; Lum. lumen; mcl. muscle layer; N. nucleus; Nb. nephric bud; Nl. nucleolus; Nt. nephric tubule; PE. pronephric epithelium; PMb. pronephric membrane; Ps. pronephric space; sb. striated border; sEHG. syncytial epithelium of the hindgut.

PLATE VIII



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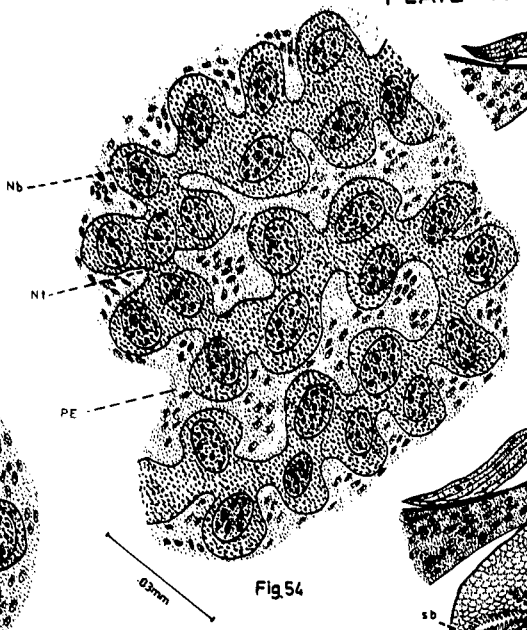


Fig.54

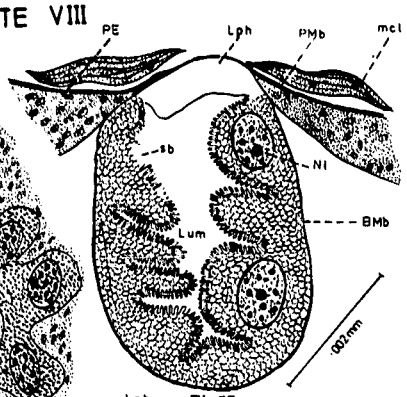


Fig.55

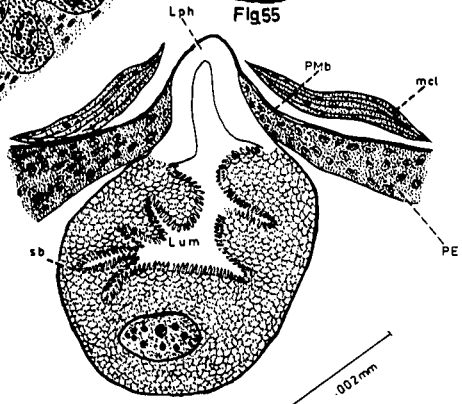


Fig.56

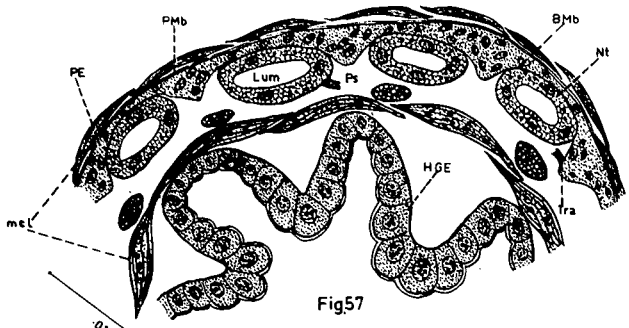


Fig.57

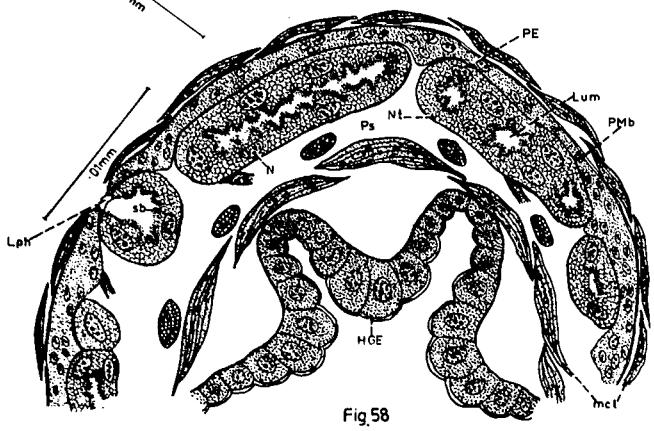


Fig.58

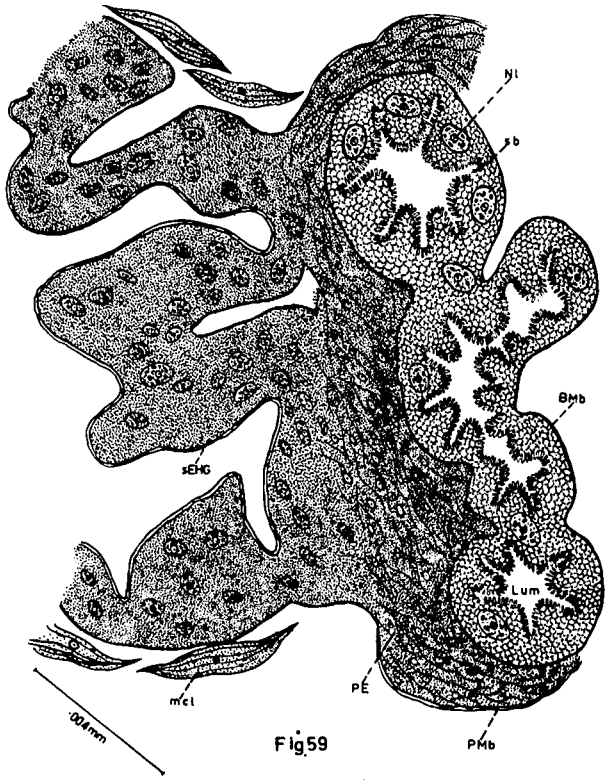


Fig.59

PLATE IX

Fig. 60. L.S. showing various components of the cryptonephric complex in C. septumpunctata.

Fig. 61. The junction between the ampulla and the gut in C. festiva.

Fig. 62. L.S. showing malpighian tubule opening into the lumen of the gut in C. septumpunctata.

Fig. 63. Displacement of the malpighian tubule in C. festiva.

Fig. 64. T.S. of the first region of the malpighian tubule in C. festiva.

Fig. 65. T.S. of the third region of the malpighian tubule in C. festiva.

Fig. 66. T.S. of the second region of the malpighian tubule in C. festiva.

Fig. 67. T.S. of the ampulla just before entering into the gut wall in C. festiva.

AINt. anterior intestine; AMal. ascending limb of the malpighian tubule; Amp. ampulla; BMb. basement membrane; DMal. descending limb of the malpighian tubule; FcMb. infoldings of the cell membrane, HG. hind gut; HGE. hindgut epithelium; In. intima; Lum. lumen; Mal. malpighian tubule; mcl. muscle layer; MG. midgut; MGE. midgut epithelium; N. nucleus; Nl. nucleolus; Nt. nephric tubule; PE. pronephric epithelium; Pl. peritoneal layer; PMb. pronephric membrane; RE. rectal epithelium; Rect. rectum; Res. reservoir; sb. striated border; VentVlv. ventricular valve.

PLATE IX

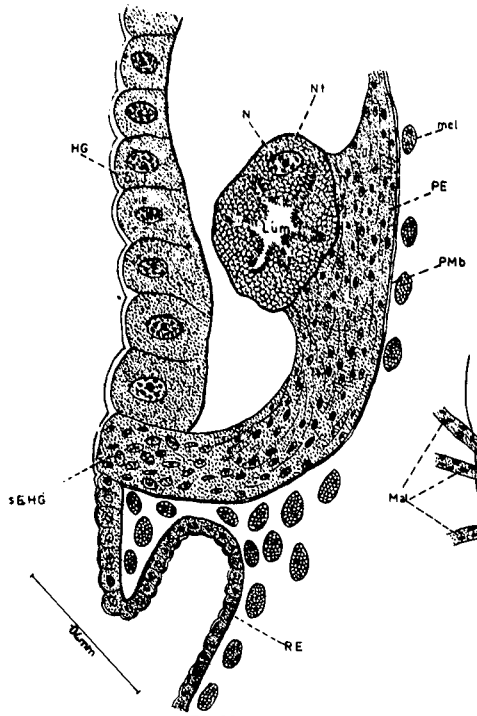


Fig.60

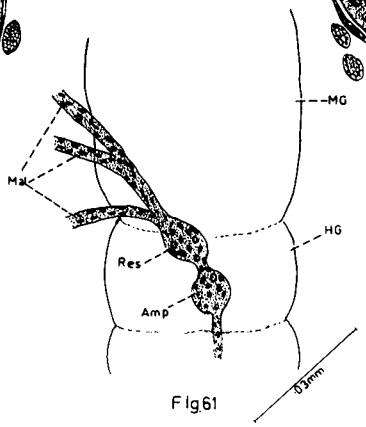


Fig.61

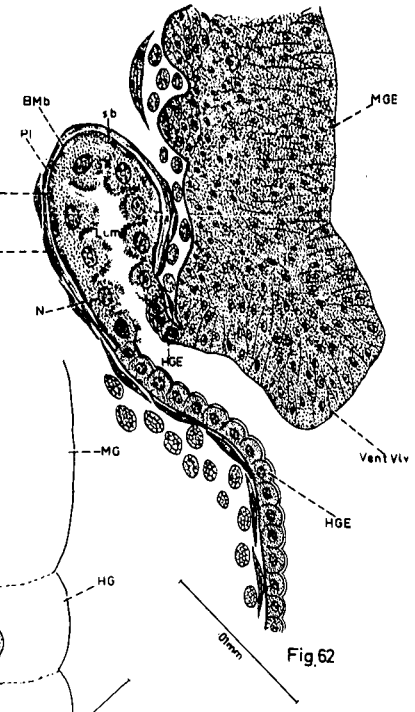


Fig.62

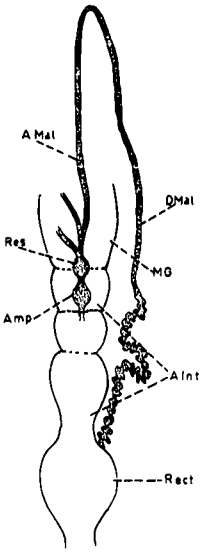


Fig.63

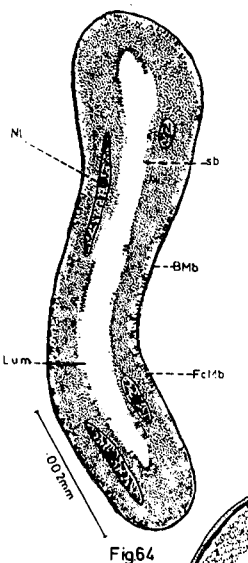


Fig.64

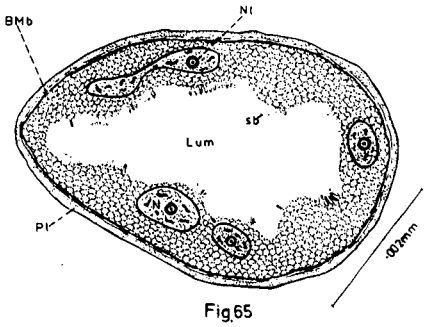


Fig.65

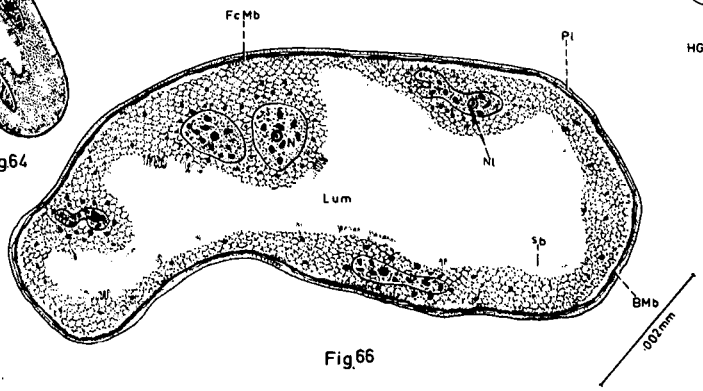


Fig.66

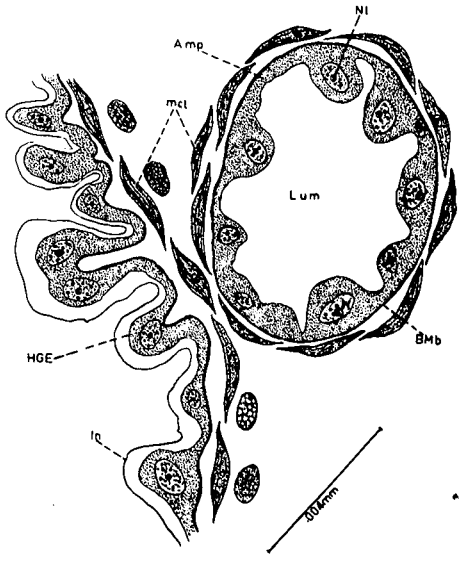


Fig.67

PLATE X

Fig. 68. T.S. of the root of the ampulla lying in the gut wall between the muscle layer and the epithelium in C. festiva.

Fig. 69. T.S. of the reservoir of the ampulla in C. festiva.

Fig. 70. Oblique section of the root of the ampulla showing its opening into the gut lumen in C. festiva.

Fig. 71. L.S. showing the various components of the crypto-nephric complex in C. festiva.

Fig. 72. Displacement of malpighian tubules along the gut in D. cucurbitae.

Fig. 73. T.S. of proximal region of the left malpighian tubule in D. cucurbitae .

Amp. ampulla; ANt. anterior portion of the nephric tubule; BMb. basement membrane; CD. common duct; FcMb. infoldings of the cell membrane; FG. foregut; GR. granules; HGE. hind gut epithelium; In. intima; iPE. inner pronephric epithelium; LMal. left malpighian tubule; Lum. lumen; mcl. muscle layer; MG. midgut; N. nucleus, Nl. nucleolus; oPE. outer pronephric epithelium; Pl. peritoneal layer; PNt. posterior portion of the nephric tubule; RE. rectal epithelium; Rect. rectum; RMal. right malpighian tubule; sb. striated border; Tra. tracheole.

PLATE

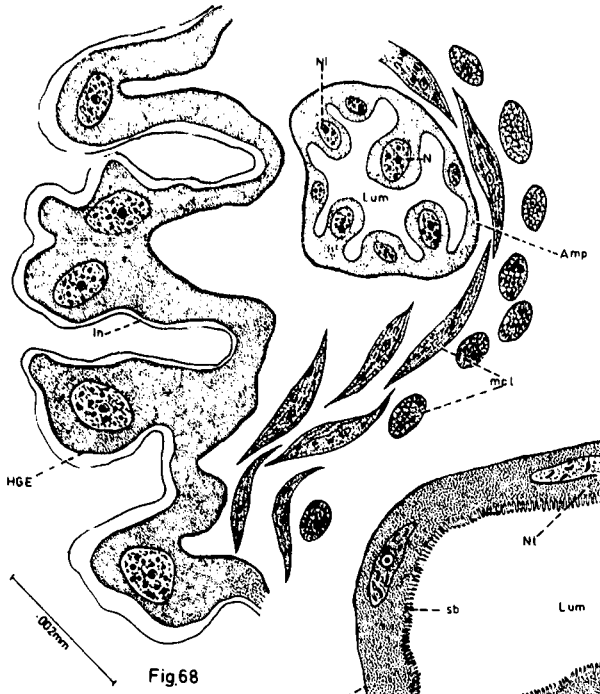


Fig.68

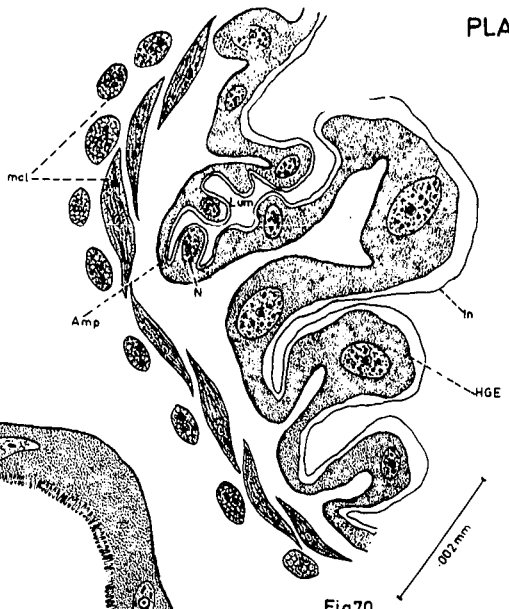


Fig.70

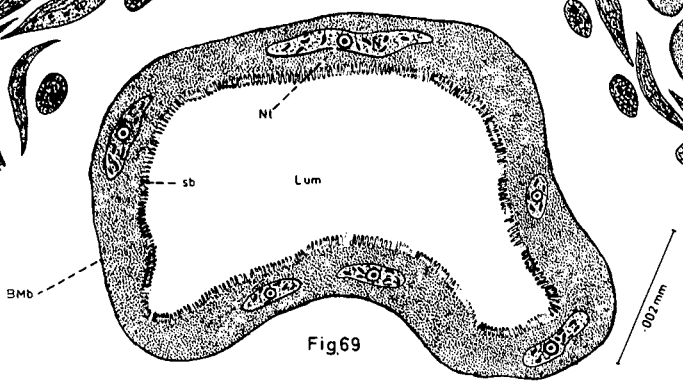


Fig.69

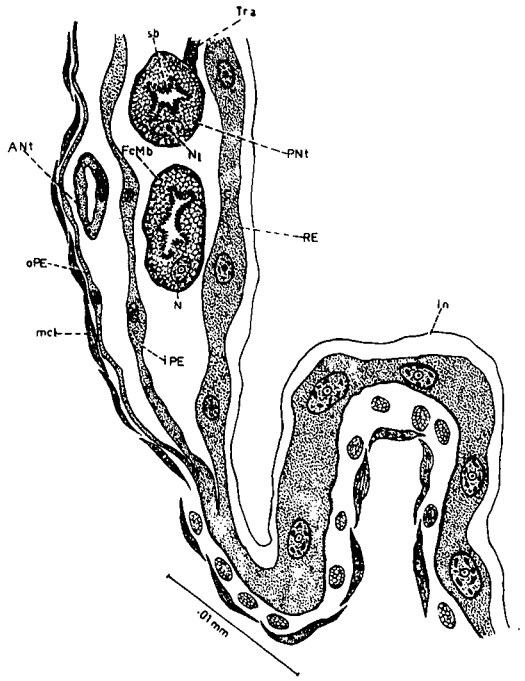


Fig.71

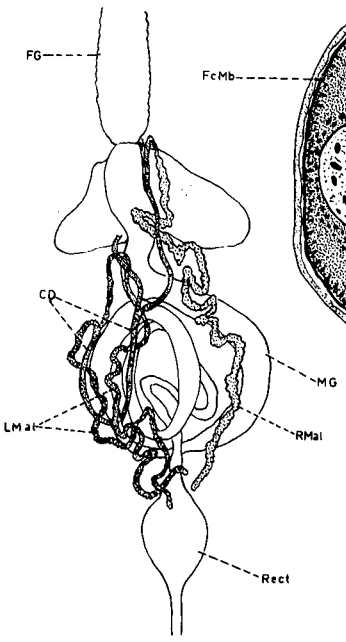


Fig.72

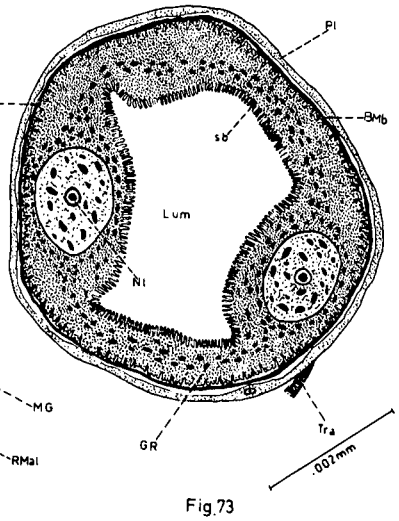


Fig.73

PLATE XI

Fig. 74. The junction between the common duct of the tubule and the gut in *D. cucurbitae*.

Fig. 75. T.S. of the second region of the left tubule of *D. cucurbitae*.

Fig. 76. T.S. of the transitional region of the right tubule of *D. cucurbitae*.

Fig. 77. L.S. of the third region of the right tubule of *D. cucurbitae*.

Fig. 78. T.S. of the distal region of the common duct of the tubule in *D. cucurbitae*.

Fig. 79. L.S. of the common duct opening into the lumen of the gut in *D. cucurbitae*.

Fig. 80. T.S. of the proximal region of the common duct of the tubule in *D. cucurbitae*.

Bm. basement membrane; CD. common duct; FcMb. infoldings of the cell membrane; HG. hind gut; HGE. hind gut epithelium; Ic. interstitial cell; In. intima; Lum. lumen; MG. mid gut; MGE. midgut epithelium; N. nucleus; Nl. nucleolus; Pl. peritoneal layer; sb. striated border; VentVlv. ventricular valve.

PLATE XI

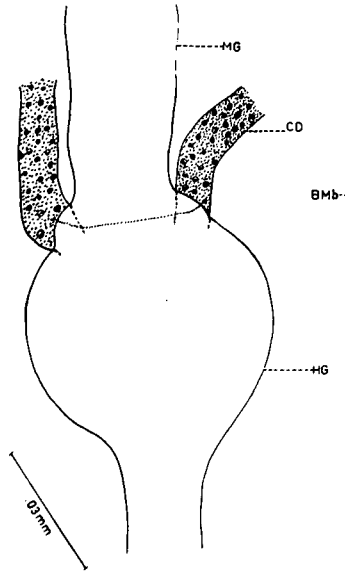


Fig.74

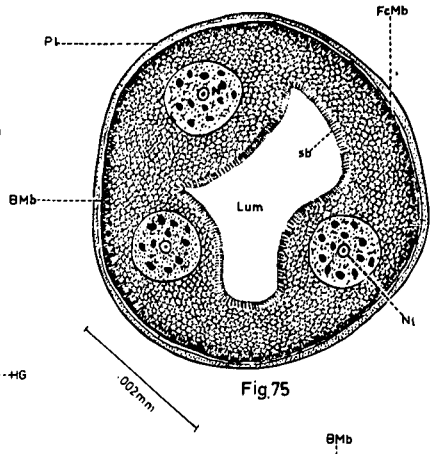


Fig.75

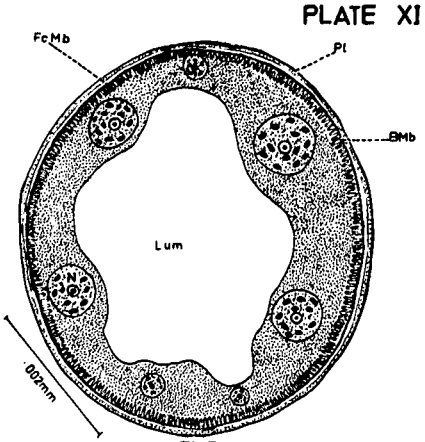
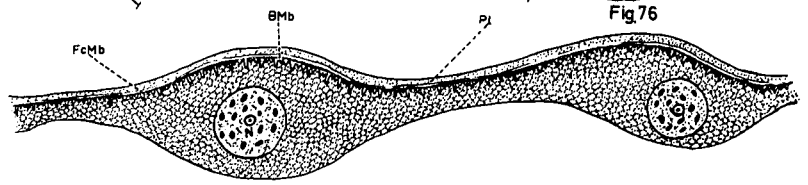


Fig.76



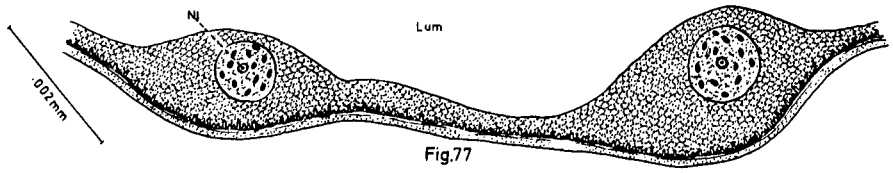


Fig.77

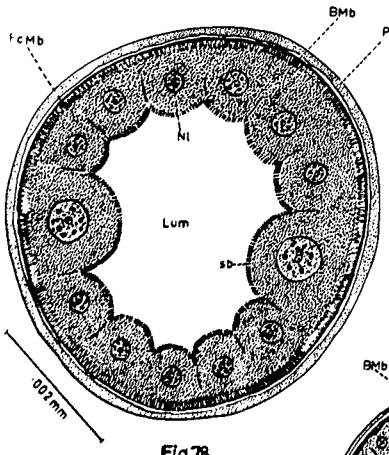


Fig.78

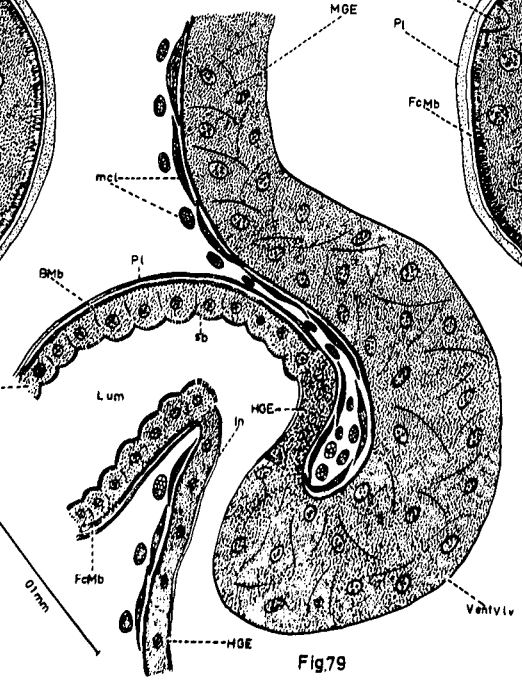


Fig.79

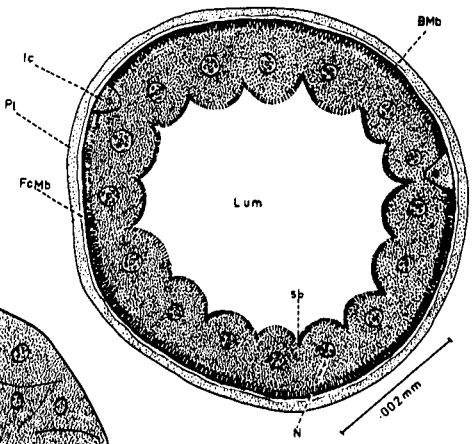


Fig.80

PLATE XII

Fig. 81. Displacement of malpighian tubule along with the gut in V. bicolor.

Fig. 82. The junction between the malpighian tubule and the gut in V. bicolor.

Fig. 83. T.S. of the proximal portion of the first region of the malpighian tubule in V. bicolor.

Fig. 84. T.S. of the intermediate portion of the first region of the malpighian tubule in V. bicolor.

Fig. 85. T.S. of the distal portion of the first region of the malpighian tubule in V. bicolor.

Fig. 86. T.S. of the second region of the malpighian tubule in V. bicolor.

Fig. 87. T.S. of the third region of the malpighian tubule in V. bicolor.

Fig. 88. L.S. of the basal portion of the tubule showing its opening into the gut lumen.

BMb. basement membrane; FcMb. infoldings of the bell membrane; GR. granule; HG. hindgut; HGE. hindgut epithelium; Lum. lumen; Mal. malpighian tubule; mcl. muscle layer; MG. midgut; N. nucleus; Nl. nucleolus; Pl. peritoneal layer; ProcVlv. proctodaeal valve; sb. striated border.

PLATE XII

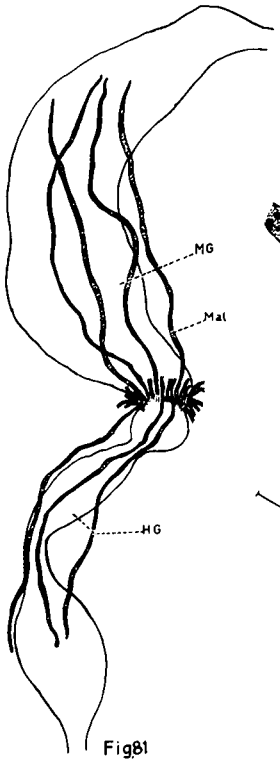


Fig.81

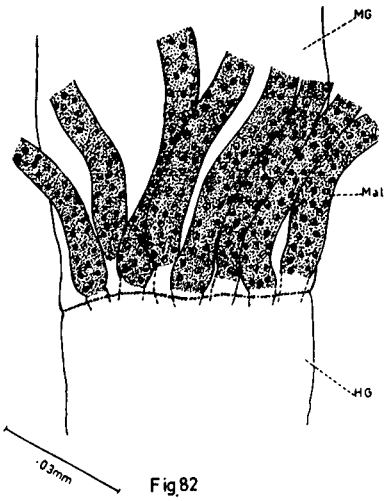


Fig.82

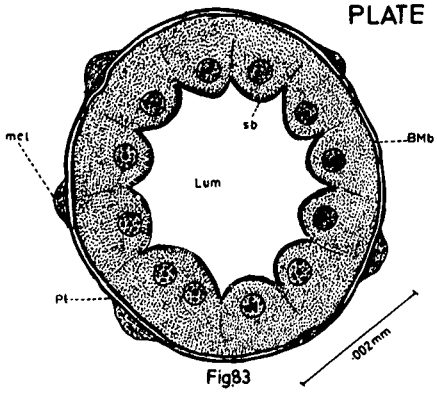


Fig.83

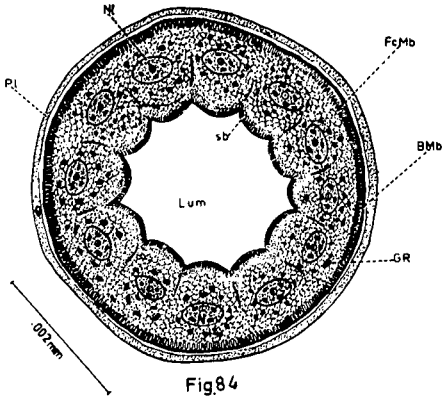


Fig.84

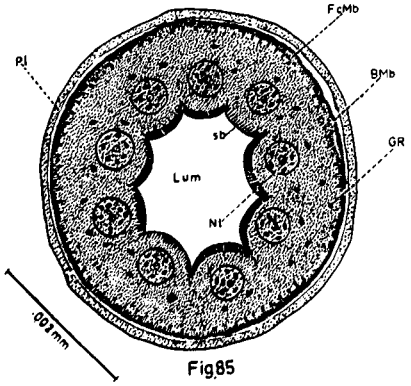


Fig.85

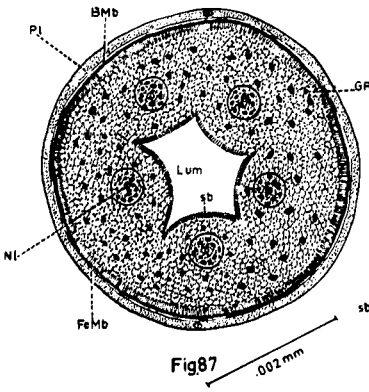


Fig.87

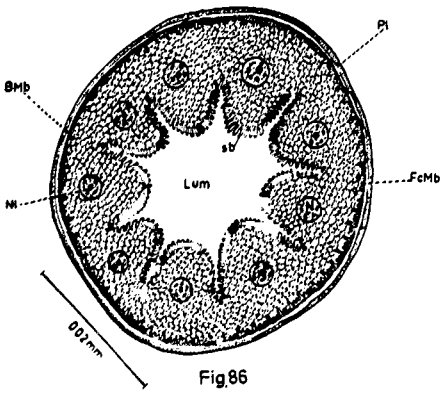


Fig.86

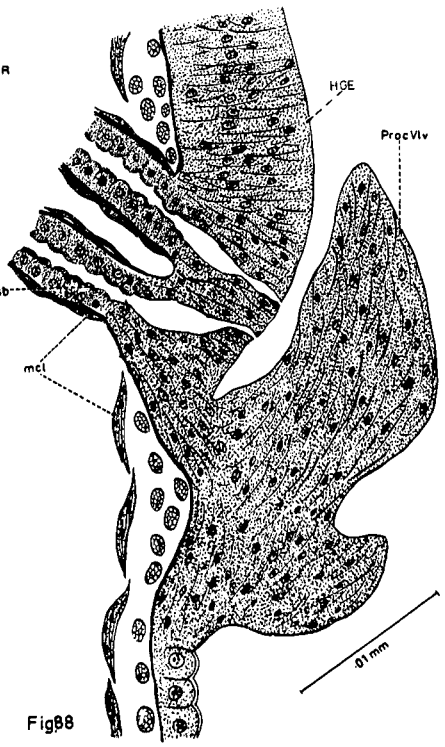


Fig.88

PLATE XIII

Fig. A. Calibration curve for the estimation of total phosphorus.

Fig. B. Calibration curve for the estimation of total nitrogen.

Fig. C. Weight of excreta in *L. maculatus*.

●———● Normal

●.....● Experimental

Fig. D. Weight of excreta in *M. pustulata*.

●.....● Normal

●———● Experimental

Fig. E. Body weight of *L. maculatus*

●.....● Normal

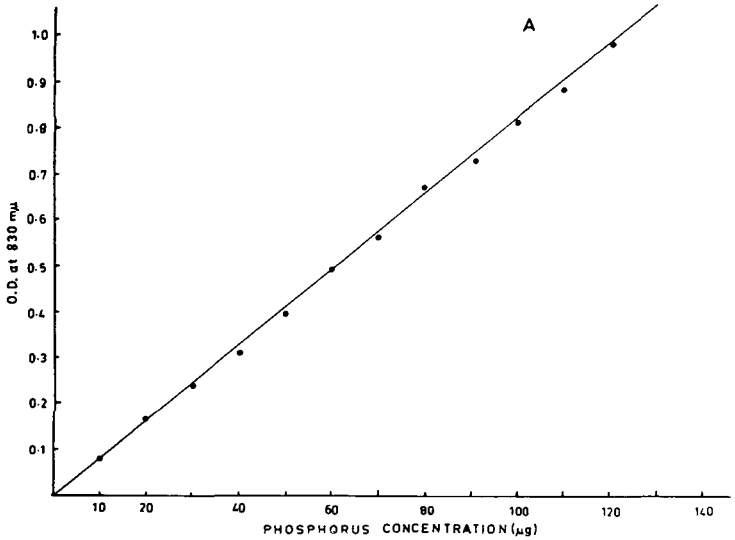
●———● Experimental

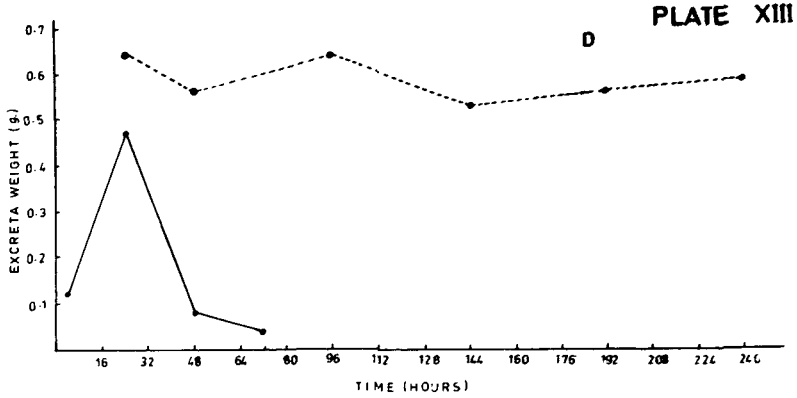
Fig. F. Body weight of *M. pustulata*.

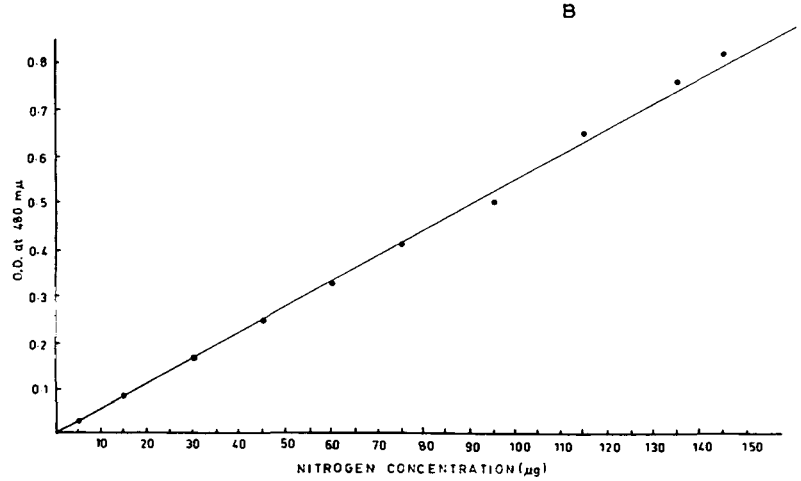
●.....● Normal

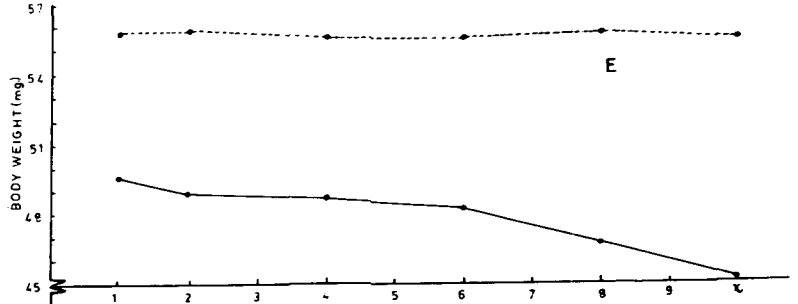
●———● Experimental

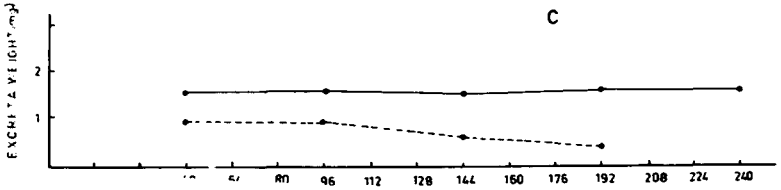
PLATE XIII


A
O.D. at 830 mμ
PHOSPHORUS CONCENTRATION (μg)


D
EXCRETA WEIGHT (g)
TIME (HOURS)


B
O.D. at 480 mμ
NITROGEN CONCENTRATION (μg)


E
BODY WEIGHT (mg)


C

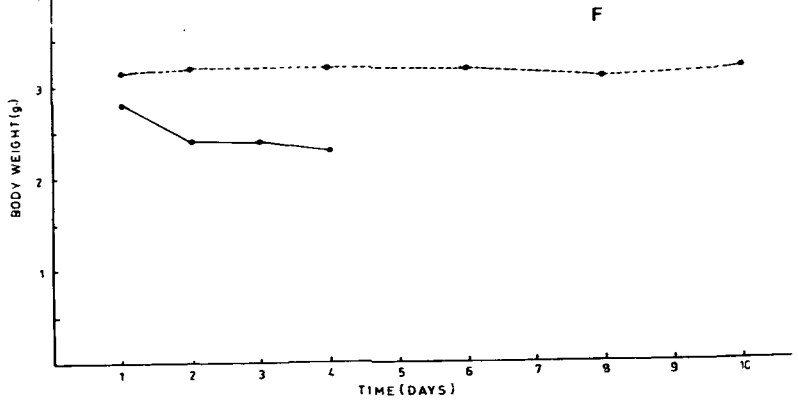

F
BODY WEIGHT (g)
TIME (DAYS)

PLATE XIV

Fig. A. Nitrogen concentration in the tubules of *M. pustulata*.

o.....o Normal

Fig. B. Nitrogen concentration in the malpighian tubules of *L. maculatus* and *M. pustulata* under experimental condition and *L. maculatus* under normal fed condition.

●——● Experimental *M. pustulata*
●.....● Experimental *L. maculatus*
o——o Normal fed *L. maculatus*

Fig. C. Nitrogen concentration in the blood of *M. pustulata*.

●.....● Normal
o——o Experimental

Fig. D. Nitrogen concentration in the blood of *L. maculatus*.

●.....● Normal
o——o Experimental

Fig. E. Phospholipid concentration in the tubules of *M. pustulata*.

●.....● Normal
o——o Experimental

Fig. F. Phospholipid concentration in the blood of *M. pustulata*.

●.....● Normal
o——o Experimental

Fig. G. Phospholipid in the blood of *L. maculatus*.

o——o Normal
●.....● Experimental

Fig. H. Phospholipid in the tubules of *L. maculatus*.

●.....● Normal
●——● Experimental

PLATE XIV

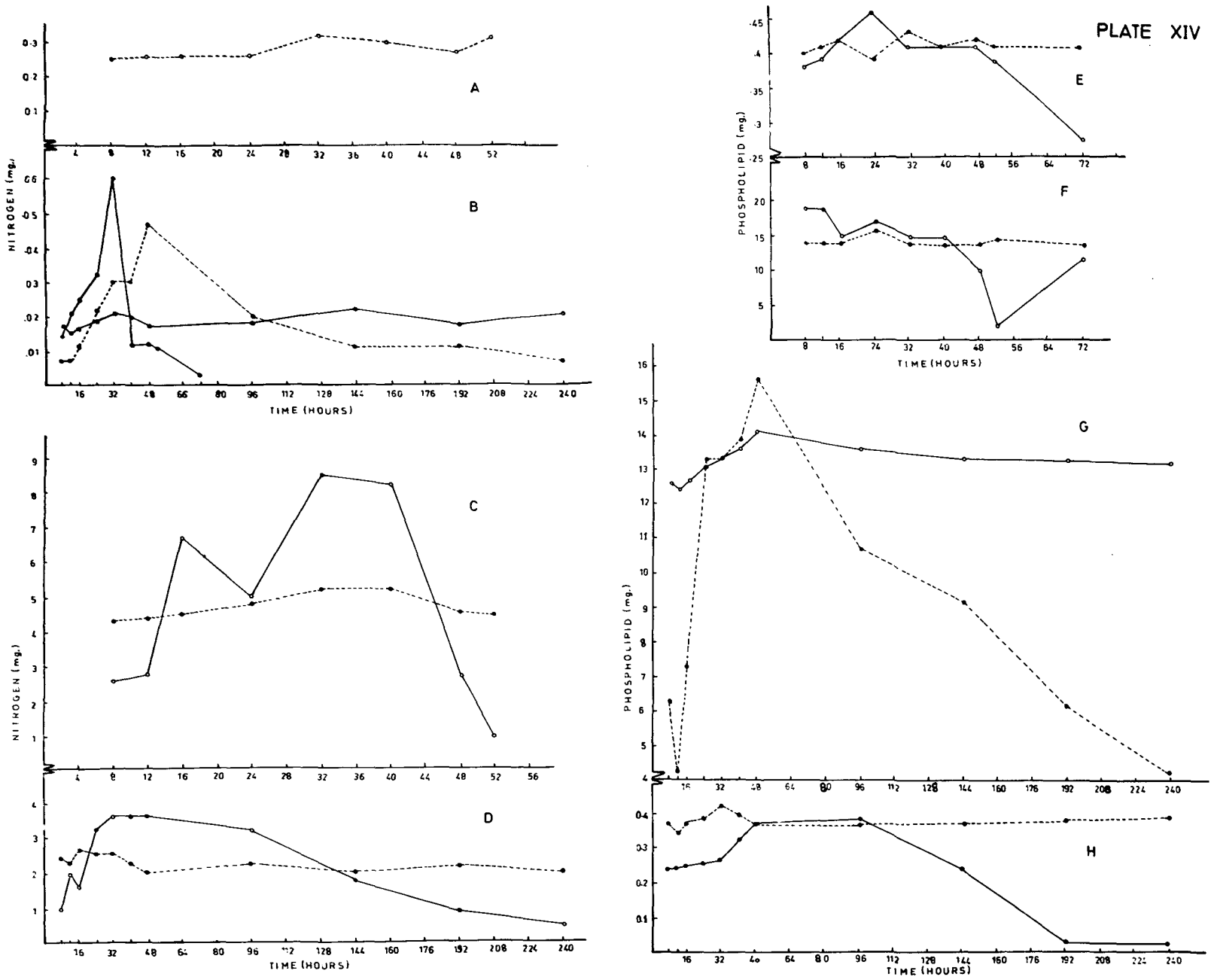


PLATE XV

Fig. A. Protein concentration in the excreta of *M. pustulata* and *L. maculatus*.

o——o Normal *L. maculatus*
●——● Experimental *L. maculatus*
o.....o Normal *M. pustulata*

Fig. B. Nitrogen concentration in the excreta of *M. pustulata* and *L. maculatus*.

o——o Normal *L. maculatus*
●——● Experimental *L. maculatus*
o.....o Normal *M. pustulata*

Fig. C. Protein concentration in the blood of *M. pustulata* and *L. maculatus*.

o——o Normal *L. maculatus*
●——● Experimental *L. maculatus*
●.....● Experimental *M. pustulata*

Fig. D. Protein concentration in the tubule of *M. pustulata*.

o——o Normal

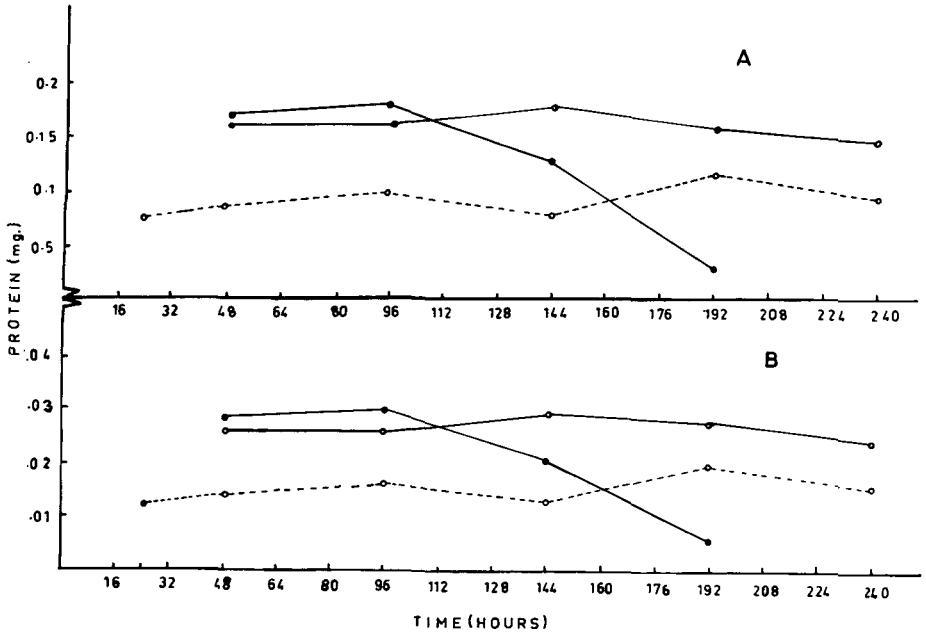
Fig. E. Protein concentration in the blood of *M. pustulata*.

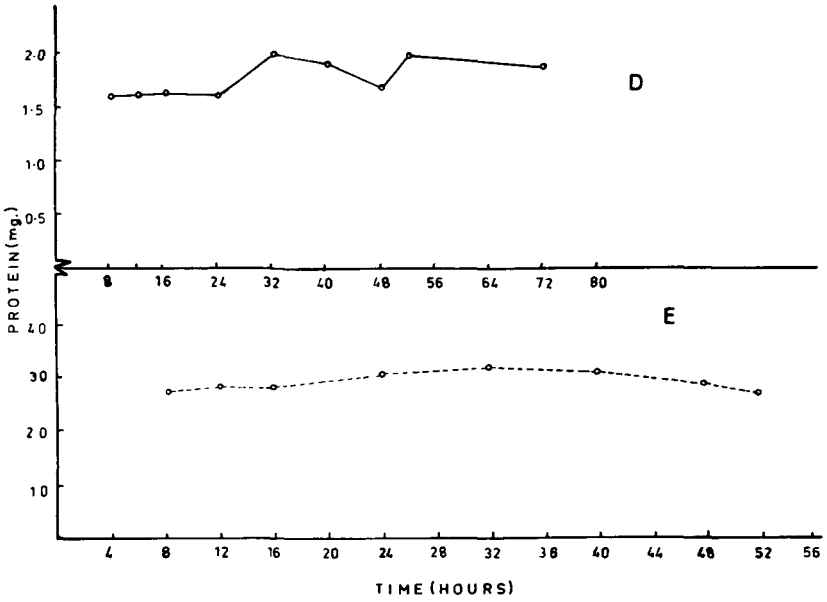
o.....o Normal

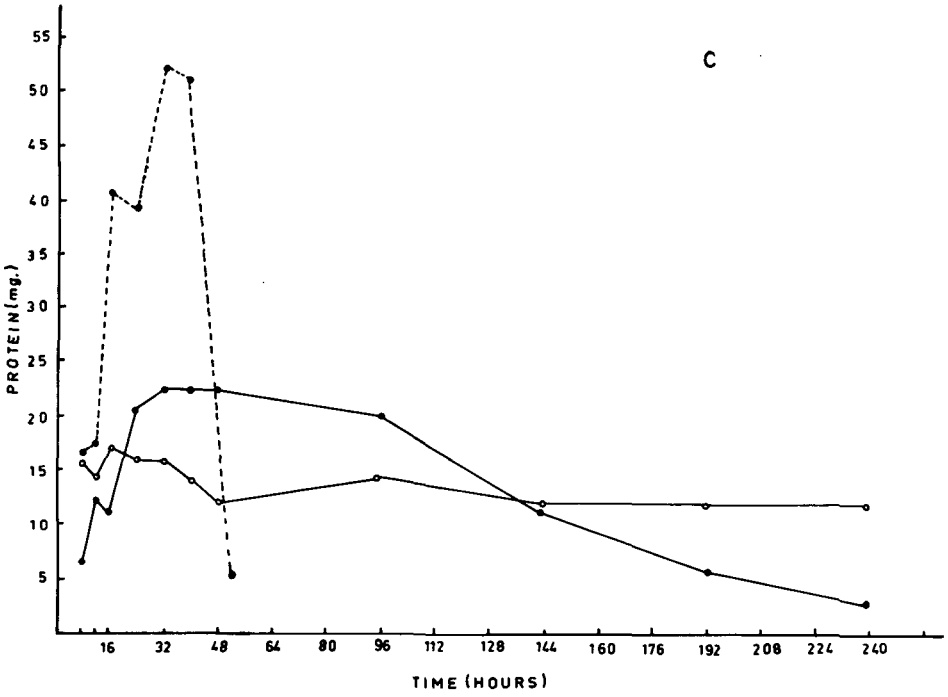
Fig. F. Protein concentration in the tubule of *L. maculatus* and *M. pustulata*.

●.....● Experimental *M. pustulata*
●——● Normal *L. maculatus*
o——o Experimental *L. maculatus*

PLATE XV


A
B
PROTEIN (mg.)
TIME (HOURS)


D
E
PROTEIN (mg.)
TIME (HOURS)


C
PROTEIN (mg.)
TIME (HOURS)

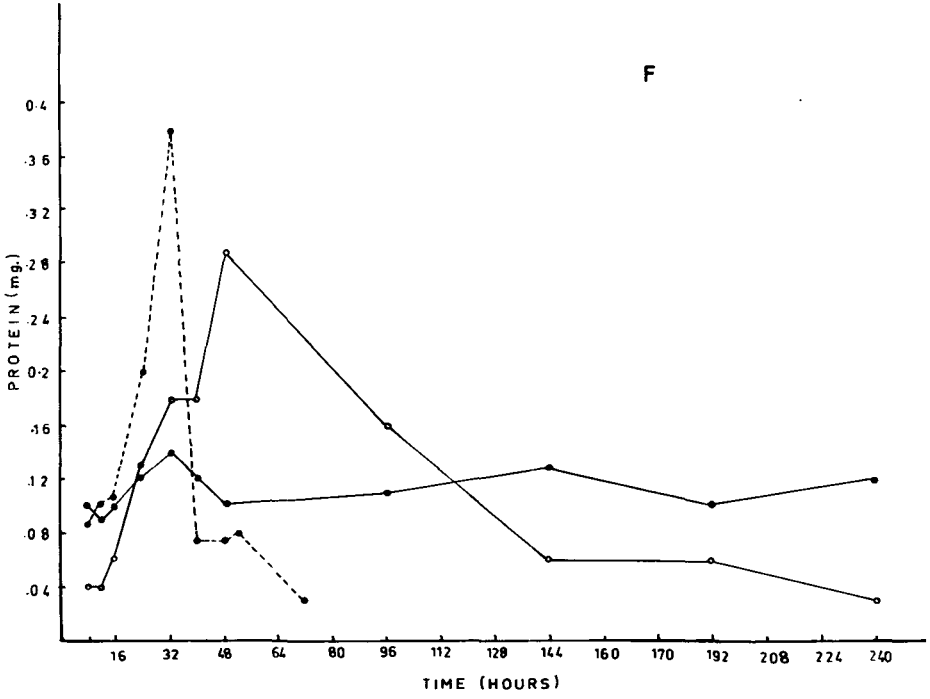

F
PROTEIN (mg.)
TIME (HOURS)

PLATE XVI

Fig. 1. T.S. of the proximal and distal regions of the free tubule of *M. pustulata* showing weak reaction to combined dialysed iron and PAS stain, at 50 hours period after feeding.

Fig. 2. T.S. of the crypto-nephric complex of *M. pustulata* showing mucoprotein substances in the nephric tubule at 8 hours period after feeding.

Fig. 3. L.S. of free tubule of *M. pustulata* showing the enlarged cell with nucleoprotein substances.

Fig. 4. T.S. of crypto-nephric complex of *M. pustulata* showing positive reaction to combined dialysed iron and PAS stain in the enlarged cells of pronephric epithelium.

Fig. 5. T.S. of free tubule of *M. pustulata* showing negative reaction to combined dialysed iron and PAS stain in the enlarged cells at 50 hours period after feeding.

Fig. 6. T.S. of crypto-nephric complex of *M. pustulata* showing weak reaction to combined dialysed iron and PAS stain at 50 hours period of starvation.

Fig. 7. T.S. of free tubule of *M. pustulata* treated treated with combined dialysed iron and PAS stain showing the presence of granules.

Fig. 8. L.S. of the free tubule of *M. pustulata* showing both normal and granulated nuclei when treated with combined dialysed iron and PAS stain at 4 hours period after feeding.

CM. cell membrane; DS. distal region; EC. enlarged cell;

EPE. enlarged pronephric epithelium; G. glycogen; GR. granules;

ML. muscle layer; MP. mucoprotein; NG. Nuclei with granules;

NN. normal nucleus; NP. nucleoprotein; NT. nephric tubule;

PE. pronephric epithelium; PS. proximal region.

plate XVI

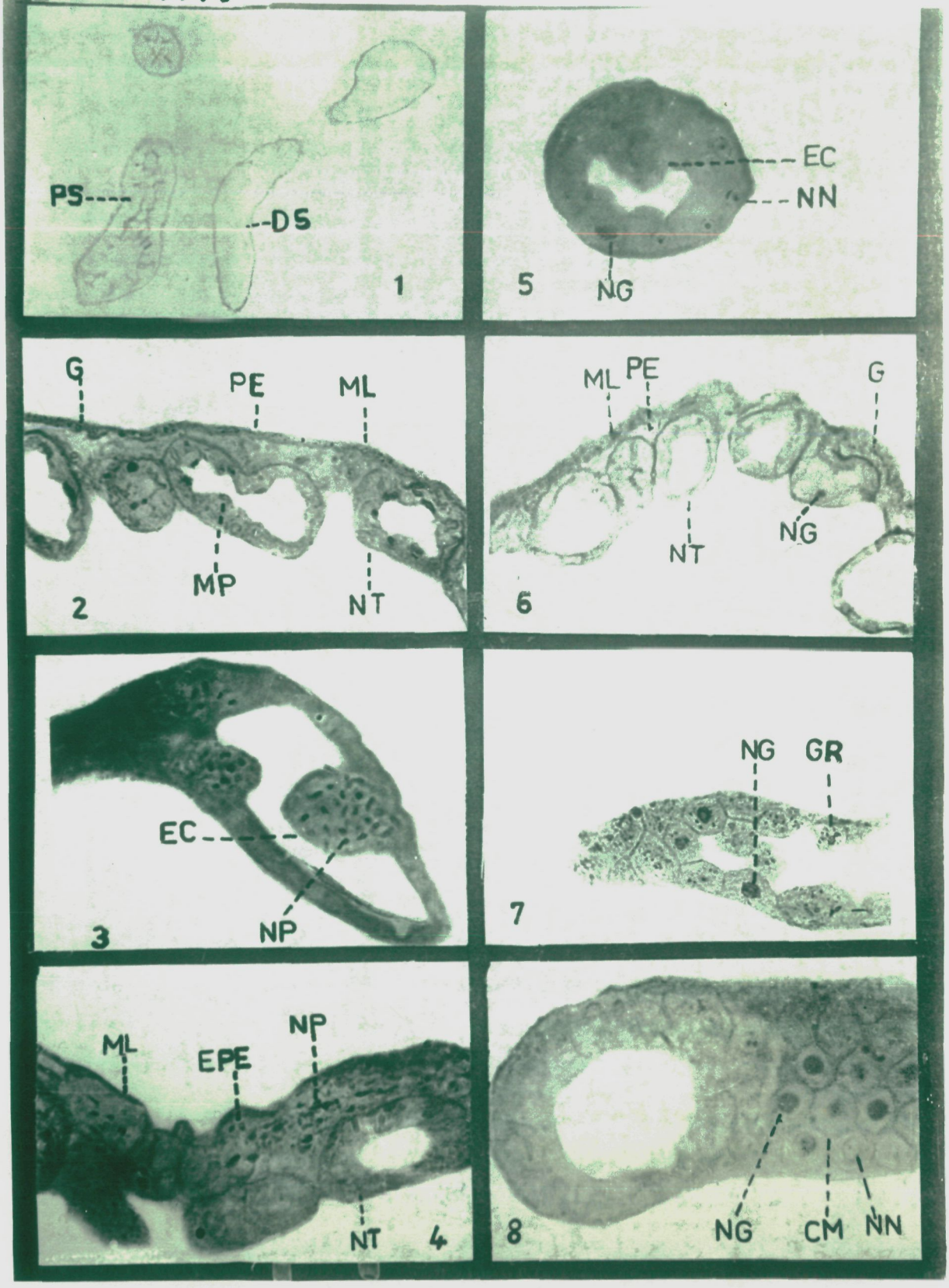

PS
DS
1
EC
NN
5
NG
G
PE
ML
MP
NT
2
ML
PE
G
NT
NG
6
EC
NP
3
NG
GR
7
ML
EPE
NP
NT
4
NG
CM
NN
8

PLATE XVII

Fig. 1. T.S. of proximal and distal regions of the free tubule of *M. pustulata* showing weak reaction to PAS stain at 50 hours period after feeding.

Fig. 2. L.S. of proximal region of the free tubule of *M. pustulata* showing the mucoprotein substances in the cytoplasma as well as the border zonea at 4 hours period after feeding.

Fig. 3. L.S. of proximal segment of free tubule of *M. pustulata* showing the PAS positive substance in the border zone at 15 hours period after feeding.

Fig. 4. T.S. of the proximal region of free tubule of *M. pustulata* showing enlarged cell with negative reaction to PAS stain at 6 hours period after feeding.

Fig. 5. L.S. of the distal region of free tubule of *M. pustulata* showing enlarged cell with negative reaction to PAS stain at 6 hours period after feeding.

Fig. 6. L.S. of the distal region of free tubule of *M. pustulata* showing positive reaction to combined dialysed iron and PAS stain in the apical half of the central zone at 15 hours period after feeding.

Fig. 7. T.S. of the distal region of free tubule of *M. pustulata* showing PAS positive substances at 22 hours period after feeding.

Fig. 8. T.S. of the free tubule of *M. pustulata* showing the granulated nuclei when treated with PAS stain.

BM. basement membrane; DS. distal region; EC. enlarged cell;

G. glycogen; MP. mucoprotein; NG. nuclei with granules;

PS. proximal region; SBR. striated border.

PLATE XVIII

Fig. 1. T.S. of the proximal region of free tubule of M. pustulata showing the presence of very weak PAS positive reaction in the border zone as well as basement membrane at 2 hours after feeding.

Fig. 2. T.S. of the distal region of free tubule of M. pustulata showing negative reaction to PAS stain at 2 hours period after feeding.

Fig. 3. T.S. of crypto-nephric complex of M. pustulata showing the presence of PAS positive substances in the muscle layer and the outer membrane of nephric tubule at 2 hours period after feeding.

Fig. 4. T.S. of free tubule of M. pustulata showing the PAS positive substances in the apical half of the central zone of distal region and in the border zone of proximal region at 15 hours period after feeding.

Fig. 5. T.S. of distal segment of the free tubule of M. pustulata showing the glycogen positive substance when treated with Best carmine stain in the apical half of the central zone at 15 hours period after feeding.

Fig. 6. Sections of free tubule of M. pustulata showing the digestion of PAS positive substances after treatment with saliva.

Fig. 7. T.S. of crypto nephric complex of M. pustulata showing the presence of PAS positive substances at 15 hours period in the muscle layer.

Fig. 8. T.S. of crypto-nephric complex of M. pustulata showing the digestion of PAS positive substances after treatment with saliva.

BM. basement membrane; DS. distal region; G. glycogen; GD. glycogen digested area; ML. muscle layer; PS. proximal region; SBR. striated border.

Plate XVIII

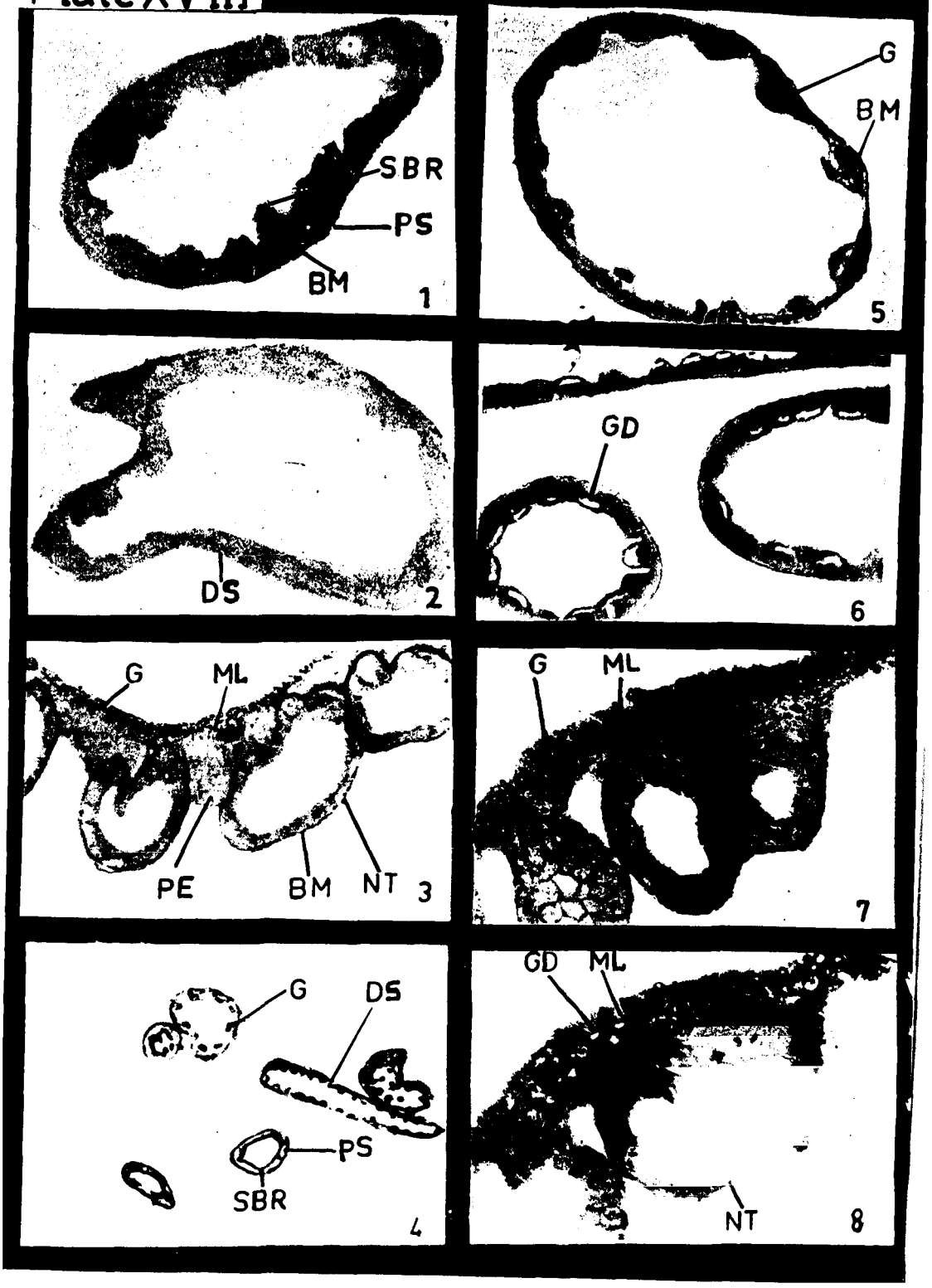


PLATE XIX

Fig. 1. T.S. of the free tubule of *M. pustulata* showing weak HgBPB positive reaction for the cytoplasm but the basal membrane shows moderate reaction at 4 hours period after feeding.

Fig. 2. Sections of free tubule of *M. pustulata* showing strong HgBPB reaction at 8 hours period after feeding.

Fig. 3. T.S. of crypto-nephric complex of *M. pustulata* showing weak HgBPB reaction at 50 hours period after feeding.

Fig. 4. T.S. of crypto-nephric complex of *M. pustulata* showing strong HgBPB reaction at 8 hours after feeding.

Fig. 5. L.S. of free tubule of *M. pustulata* showing a negative reaction to HgBPB in the apical half of the central zone of cytoplasm at 15 hours period after feeding.

Fig. 6. T.S. of malpighian tubule of *L. maculatus* showing weak HgBPB reaction in starvation.

Fig. 7. Sections of malpighian tubule of *L. maculatus* showing strong HgBPB reaction in fed insect.

Fig. 8. Sections of the third region of malpighian tubule of *L. maculatus* showing HgBPB positive granules at 4 hours period after feeding.

BM. basement membrane; N. nucleus; NT. nephric tubule;

PE. pronephric epithelium; PNR. HgBPB negative region;

PPS. HgBPB positive granule.

plate XIX

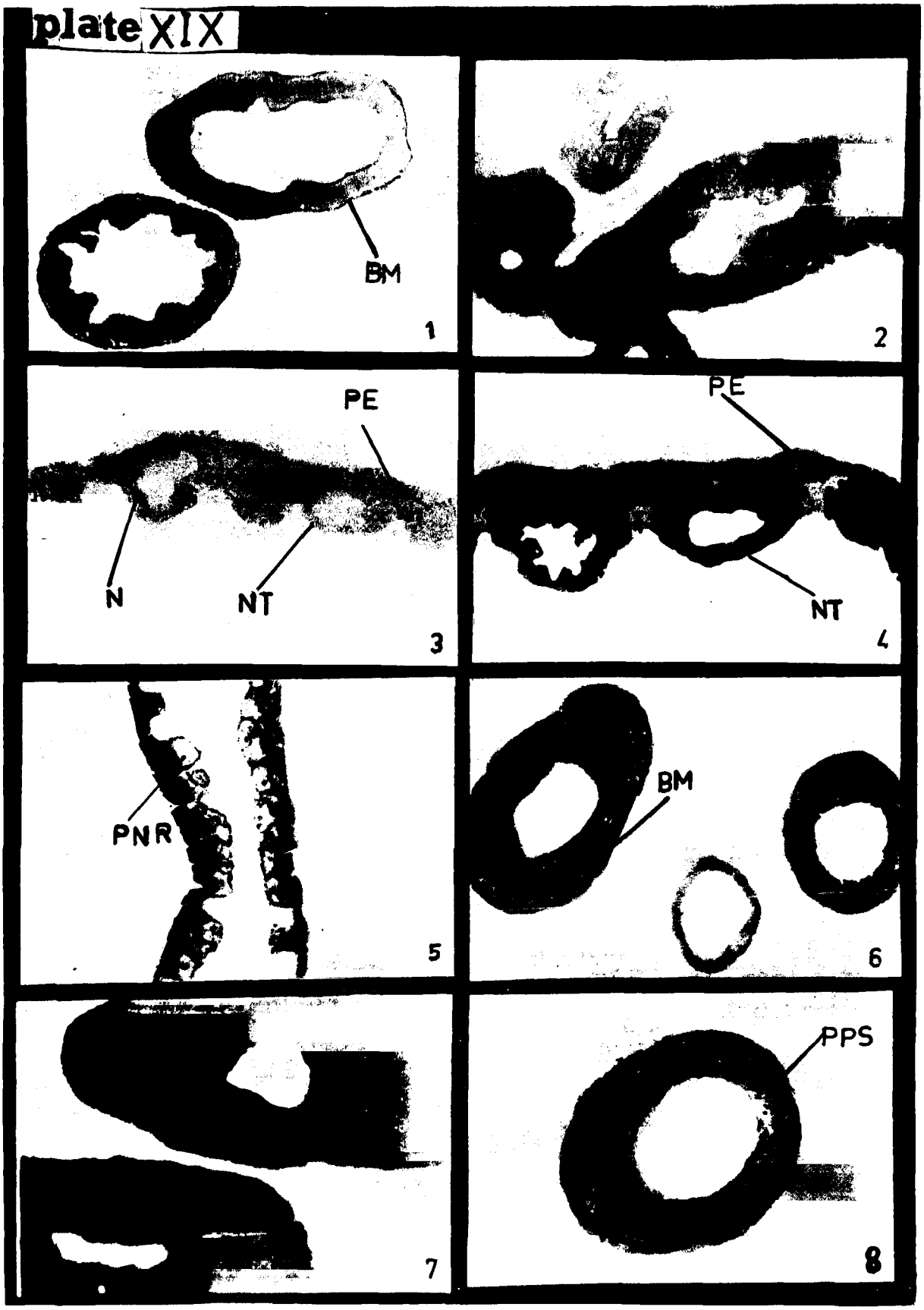


PLATE XX

Fig. 1. L.S. of free tubule of M. pustulata showing Feulgen positive substance in fed insect.

Fig. 2. T.S. of crypto-nephric complex of M. pustulata showing Feulgen positive substance in fed insects.

Fig. 3. T.S. of free tubule of M. pustulata showing the enlarged cell with negative Feulgen reactions.

Fig. 4. T.S. of crypto-nephric complex of M. pustulata showing enlarged pronephric epithelium with weak Feulgen positive reaction.

Fig. 5. T.S. of free tubule of M. pustulata showing weak Feulgen positive reaction and the nuclei filled with gramules during starvation.

Fig. 6. T.S. of crypto-nephric complex showing the nuclei of nephric tubule of M. pustulata filled with granules during starvation.

Fig. 7. T.S. of the proximal region of the free tubule of M. pustulata showing RNA positive granules in fed insect.

Fig. 8. L.S. of the distal region of the free tubule of M. pustulata showing RNA positive granules both in the nuclei and cytoplasm in fed condition.

DPS. Feulgen positive substance; EC. enlarged cell;

EPE. enlarged pronephric epithelium; GN. granulated nuclei;

NT. nephric tubule; RNS. RNA positive substance.

plate XX

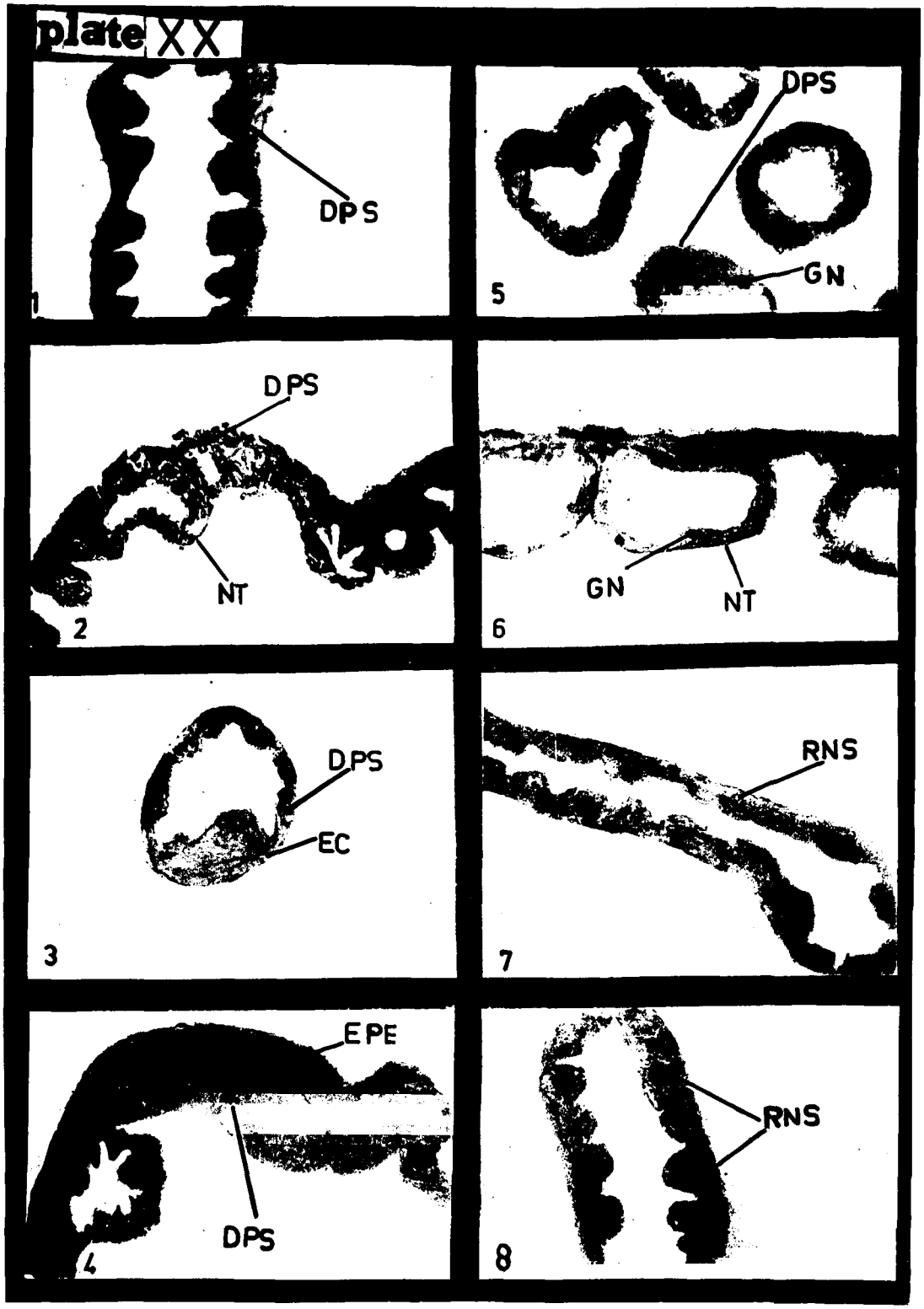


PLATE XXI

Fig. 1. Sections of proximal region of free tubule of M. pustulata showing the RNA positive substances in the cytoplasm during starvation.

Fig. 2. T.S. of crypto-nephric complex of M. pustulata showing the RNA positive substances at 8 hours period after feeding.

Fig. 3. T.S. of free tubule of M. pustulata showing RNA positive substances in the enlarged cell.

Fig. 4. T.S. of crypto-nephric complex of M. pustulata showing RNA positive substances in the enlarged pronephric epithelium.

Fig. 5. Sections of distal region of free tubule of M. pustulata showing negative reaction to RNA stain at 46 hours period after feeding.

Fig. 6. T.S. of crypto-nephric complex of M. pustulata showing moderate RNA positive reaction in the basement membrane of nephric tubule during starvation.

Fig. 7. T.S. of crypto-nephric complex of M. pustulata showing strong RNA positive reaction in the nucleus of leptophragma.

Fig. 8. L.S. of free tubule of M. pustulata showing weak HgBPB reaction in the basement membrane at 50 hours period after feeding.

BM. basement membrane; DS. distal region; EC. enlarged cell; EPE. enlarged pronephric epithelium; LP. leptophragma; RNM. membrane showing RNA positive reaction; RNS. RNA positive substance.

plate XXI

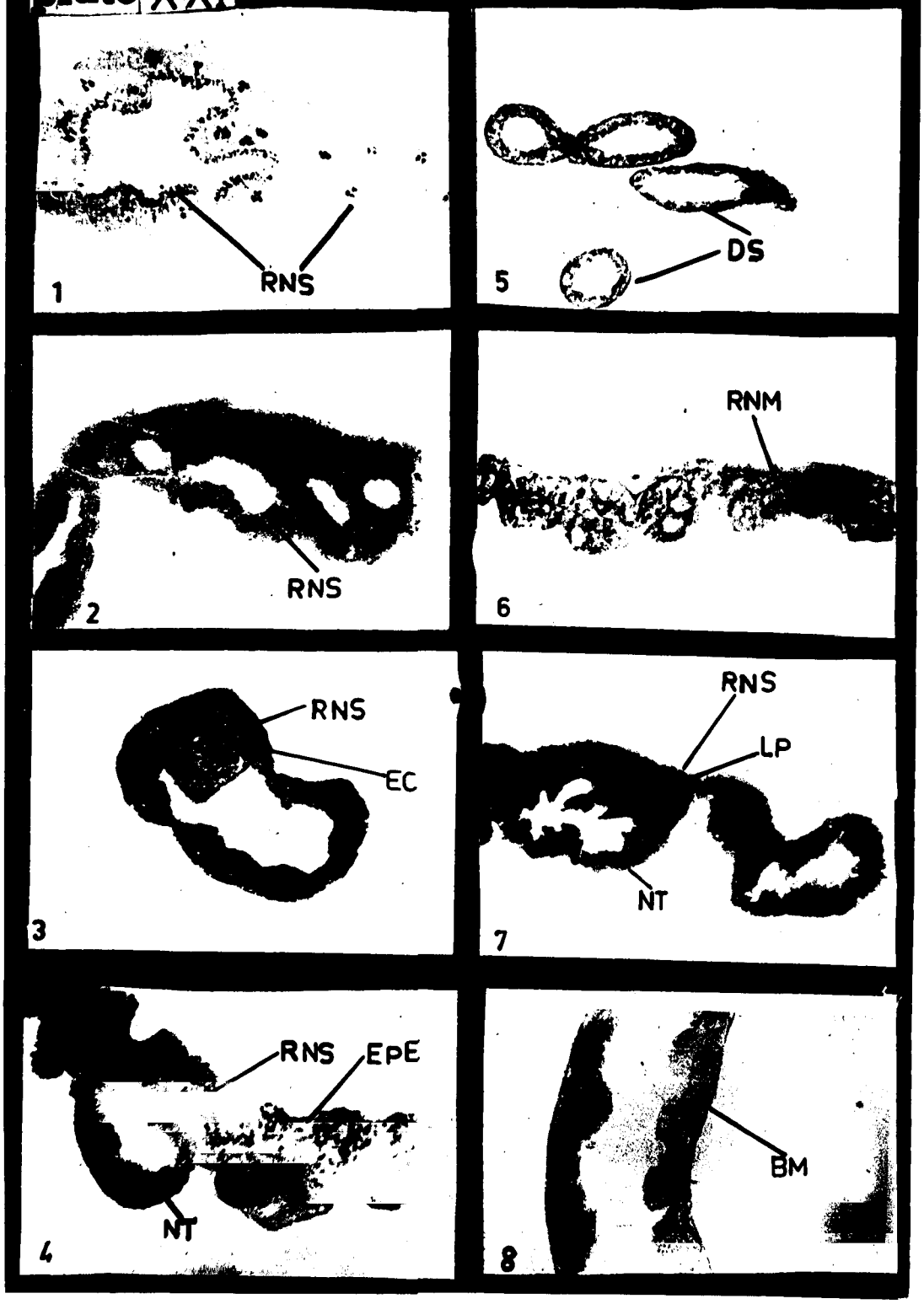


PLATE XXII

Fig. 1. T.S. of the second region of malpighian tubule of L. maculatus showing PAS positive substances in the apical part of the central zone at 2 hours period after feeding.

Fig. 2. Sections of the first and third regions of malpighian tubule of L. maculatus showing PAS positive substances at 2 hours period after feeding.

Fig. 3. T.S. of the second region of the malpighian tubule of L. maculatus showing PAS positive substances in the apical half of the central zone at 4 hours period after feeding.

Fig. 4. Sections of malpighian tubule of L. maculatus showing the PAS positive substances in the apical half of the first region and negative reaction to PAS stain in the third region except for border zone at 4 hours period after feeding.

Fig. 5. T.S. of the second region of the malpighian tubule of L. maculatus showing PAS positive substances in the basal zone at 8 hours period after feeding.

Fig. 6. L.S. of the third region of the malpighian tubule of L. maculatus showing PAS positive substances at 10 hours period after feeding.

Fig. 7. T.S. of the malpighian tubule of L. maculatus showing the digestion of PAS positive substances after the treatment with saliva.

Fig. 8. T.S. of the malpighian tubule of L. maculatus showing the mucoprotein positive substances in fed insect.

G. glycogen; GD. glycogen digested region; MP. mucoprotein.

plate XXII

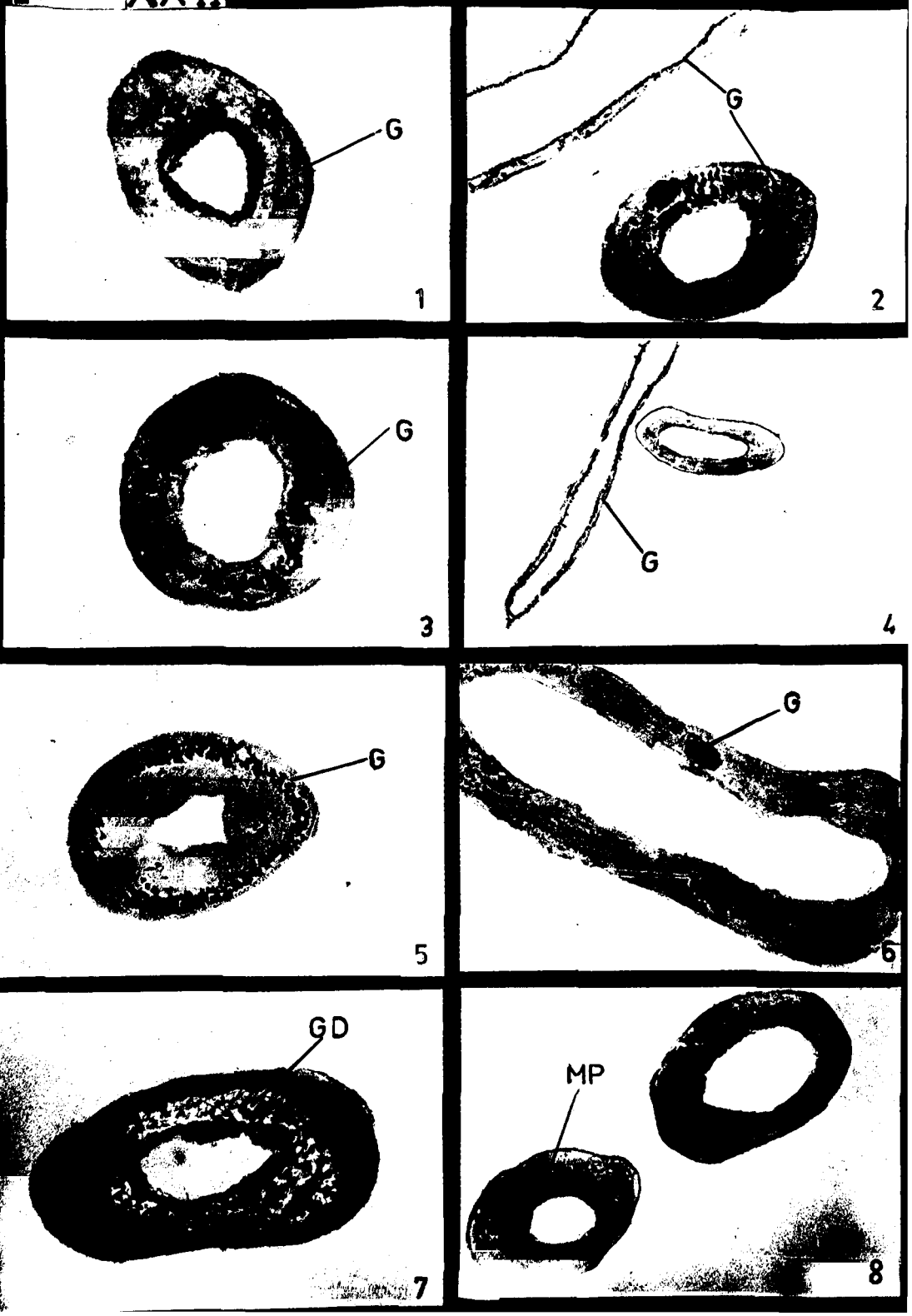


PLATE XXIII

Fig. 1. T.S. of the malpighian tubule of *L. maculatus* showing glycogen positive substances when treated with Best Carmine stain.

Fig. 2. T.S. of the malpighian tubule of *L. maculatus* showing weak Feulgen reaction during starvation.

Fig. 3. T.S. of the malpighian tubule of *L. maculatus* showing strong Feulgen reaction in the fed condition.

Fig. 4. Sections of *L. maculatus* showing weak RNA positive reaction during starvation.

Fig. 5. T.S. of malpighian tubule of *L. maculatus* showing the RNA positive substances at 4 hours period after feeding.

Fig. 6. L.S. of the third region of the malpighian tubule of *L. maculatus* showing RNA positive granules in the cytoplasm at 4 hours period after feeding.

DPS. DNA positive substance; G. glycogen;

RNS. RNA positive substance.

plate XXIII