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 viewpoint. 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), Laccotrophes maculatus F. (Hemiptera), Kylabris 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 Laectrophes 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 G. 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.