CHAPTER 7

Histology
INTRODUCTION

Digestion and absorption of nutrients to various tissues has acquired great significance in the physiology of an animal. Metabolic activities of different physiological systems affect when sufficient quantities of vitamins, minerals, amino acids, proteins and carbohydrates are not reached the tissue. Certain trace elements like Se, Ni, Zn and cobalt play a good role in the nutrition. The activities of the cell are carried out most efficiently within a narrow range of conditions. It is therefore important that the environment within the cell and in the animal in general should be kept as near optimal as possible, a process known as 'Homeostasis'. When this balance is not maintained, ultimately pathological changes and structural modification of a cell will occur. There is a persistent relationship between the histopathological changes and progression of external symptoms.

In the present investigation, an attempt has been made to study the histological aspects of some tissues such as fat body and
malpighian tubules of *Bombyx mori* L. and changes under exposure to selenium.

**NORMAL HISTOLOGY OF MALPIGHIAN TUBULES**

Excretion is the removal, from the body, of harmful products of internal metabolism. The faces are not normally included in this category. Since during its passage through the alimentary canal the food is morphologically external to the body and the process of defaecation is otherwise known as egestion. There are two main aspects of excretion namely carbonaceous and nitrogenous. Carbonaceous excretion is the removal of the carbon dioxide resulting from cellular respiration and it is carried out by the respiratory surface. Nitrogenous excretion is the removal of the wastes of nitrogenous metabolism especially that of aminoacids.

The excretory system is primarily responsible for homeostasis, which maintains a constant level of salts and water and osmotic pressure in the haemolymph. Toxic compounds absorbed from the environment are also eliminated. Some molecules entering the body from the environment may be too large or too toxic to be dealt with by the excretory system. Hence various tissues are involved in metabolizing them to detoxify or more readily extractable substances. Generally, toxic materials are eliminated from the body in the form of fluid urine, which is composed of unselective substances of the haemolymph. Useful compounds in the urine are reabsorbed and
others in excess may be added to the urine. The excretory organs of arthropods, are very varied and only in the primitive *Peripatus* is there a series of segmental paired organs comparable to annelids. In crustaceans there is a single pair of excretory organs in one segment, which may be either the antennary or maxillary segment. This suggests that with increasing efficiency it has been possible to reduce the number of excretory organs from a segmental series to one pair, but the remaining pair is not the same in all species. The opposite extreme is seen in insects which, in the adult form, are usually land animals requiring to conserve water. Excretion is performed by numerous fine out growths of the front of the hindgut called malpighian tubules. The length of the tubules ranges from 2 - 100 mm in different insects and each tubule is one cello thick with one or a few cells encircling the lumen. Each malpighian tubules is functionally divisible into two parts; the distal part absorbs from the haemocoel and secretes into the lumen a solution of sodium and potassium urates; in the proximal part the secretion of carbon dioxide causes the precipitation of the uric acid, and sodium and potassium bicarbonates are reabsorbed. The uric acid passes on to the hindgut and is voided with the faeces, thus achieving nitrogenous excretion with the minimum of water loss. Under transverse section microvilli and plasma membrane with more number of mitochondria were observed (O’ Donnel et al., 1985). Misra (1981) exhibited, gradual degeneration of cells along with its nuclei in malpighian tubules of *Hieroglyphus nigroreletus* under stress conditions.
NORMAL HISTOLOGY OF FAT BODY

The insect fat body is the principal organ of intermediary metabolism site for the requirement of the physiological activity and is similar to the liver of mammals in function.

The fat body is derived from mesoderm of the coelomic sac and composed of fat contained cells and its surface is covered by thin membrane. They are almost circular in shape during young ages and become multiangular in the older larval stage. It has one circular nucleus and some times more than two nuclei. Fat body principally has the trophocytes and in addition, it may have urate cells, hemoglobin, tracheal cells, mycetocytes and some other oenocytes. The structure of a trophocyte may vary according to its developmental
stage and nutritional status. Urate cells characteristically contain large crystalloid spherules of uric acid. Haunerland et al. (1990); and Schin et al., (1977) reported that the fat body is regionally differentiated to perform different functions such as storage in perivisceral body and synthesis in perivisceral body as observed in many insects of Lepidoptera. In accordance with metabolism, the fat body serves as the aimed tissues affected by various hormones such as juvenile hormone, moulting hormone. Fat body also serves to store fifty percent of glucose ingested to meet the requirement of energy consumption.

The fat body also takes part in the metabolism of proteins and amino acids. Synthetic ability of protein in the fat body is related to the brain and carpus cardiacum and is regulated by the neuro hormones. According to Palli and Locke (1988), 90% of total haemolymph proteins are synthesized by the fat body in larval insects. Tojo et al., (1980), studied the regulatory mechanism of protein synthesis in *Bombyx mori* L. which is under hormonal control. Juvenile hormone is the factor, which suppresses the protein synthesis, which again restarts at the end of V instar when juvenile hormone disappears from the haemolymph. Fat body accumulates carbohydrates in the form of glycogen during its active feeding periods for utilization at the time of moulting period. It was reported that the conversion of glycogen to trehalose in the fat body maintains the level of trehalose under the control of hyperglycemic hormone from the
corpora allata, which activates glycogen phosphorylase in the fat body of *Manduca sexta*. Gies et al., (1988) reported that the activity of glycogen phosphorylase in fat body was regulated by adipokinetic like hormone (AKH) under hypoglycemic condition.

The fat body also can synthesize the vitamin-C and contain a lot of xanthin oxidase and degenerate the amino acids into uric acid into body fluid to be expelled out. The arginine activity is quite high, it can be transformed into urea and ornithine by arginase. It was described that ultra structural changes in skeletal muscle and myocardium of selenium deficient pigs (Van Vleet et al., 1976 and Van Vleet et al., 1977). Muth (1963) reported the muscular degeneration or weakness in sheep due to selenium deficiency.

**RESULTS**

1. **Histopathological changes in malpighian tubules**

   Severe pathological changes were observed in the malpighian tubules of group 2 (Plate-V b) like cloudy swellings and degenerations of tubular cells. The swollen tubular cells projected into the lumen of the tubules, which reduced the size of the lumen. Some of the tubular cells exhibited granular cytoplasm. Some of the tubules were dilated and necrosis was seen in the proximal tubules.

   In group 5 (Plate VI b) also, the malpighian tubules showed noticeable changes, the nuclei of the tubular cells were not visible and
those which could be seen appear to be pyknotic (A degenerative state of the cell nucleus). The cytoplasm of many tubular cells was granular. Necrosis in proximal tubules was evident, but the extent of damage was relatively less than in group 2 (Plate V b) Silkworms. Silkworms in group 8 (Plate VII b) exhibited tubular cell oedema, round cell infiltration, tubular cell necrosis. But the extent of damage was more than that of group 4 (Plate VI b) silkworms. Silkworms in group 11 (Plate VIII b) exhibited severe degenerative changes as seen by wide spread disintegration of malpighian tubules. Necrosis of tubular cells was most pronounced. Presence of casts in tubular lumen, aggregation of chronic inflammatory cell in the interstitium was observed. In group 3 (Plate V c) silkworms showed necrosis in the tubular cells. The nuclei of the most of the oedematus tubules were not visible and appeared pyknotic and were in the process of nuclear degeneration. In group 6 (Plate VI c) silkworms, there were no appreciable changes and moderate tubular necrosis was observed. In group 9 (Plate VII c) silkworms exhibited hyperplasia of tubular tufts together with necrosis in tubular epithelium and cellular infiltration in interstitium. In group 12 (Plate VIII c) silkworms did not exhibit any pathological changes except limited signs of necrosis.

2. Histopathological Changes In Fat Bodies

Changes were observed in fat bodies of group 2 (Plate I b), group 5 (plate II b), group 8 (Plate III b) and group 11(Plate IV b) of silkworms compared to the controls. Silkworms in group 2 (Plate I b) exhibited
with hypertrophoid condition. Vacuoles appeared in the cytoplasm were somewhat less and the membranous sheath surrounding the fat cells were slightly destructed. Similarly, in group 5 (plate II b) silkworms also showed extensive cell necrosis. Hyperplasia and fatty degeneration were conspicuous. However the degenerative lesions were severe than those in group 2 (Plate I b). In group 8 (Plate III b), the fat body section showed severe structural alterations like cell necrosis of reticulo-endothelial cells and hyperplasia. Scattered area of fatty degeneration of fat body was also evident. In group 11 (Plate IV b) also appeared massive cell necrosis, cell outline disappearance, degeneration of nuclei were the most characteristic features. Hypoplasia of reticulo-endothelial cells was observed. Appreciable changes were noticed in group 3 (plate I c) pushing of the nucleus to the peripheral regions of the fat body cells, degeneration of the nucleus, pyknotic clubbing of nuclei due to cytoplasmic disappearance. In the group 6 (Plate II c) silkworms exhibited only mild structural changes, insignificant necrosis was observed. Significant changes like degeneration of nuclei, dissolution of cytoplasm was markedly noticed in group 9 (Plate III c). Whereas in group 12 (Plate IV c) no conspicuous changes were observed except a mild necrosis.

**DISCUSSION**

In a majority of insects toxic chemicals that enter the tissues are excreted often after being metabolized. The changes frequently
resulted in the compound become less toxic, but the converse is sometimes true and toxicity is enhanced. Some compounds notably those that are water soluble are metabolized to components that are subsequently incorporated into the insect's primary metabolic pathways. Most of the lipophylic substances are first converted into water soluble components and are excreted. Many different enzyme systems are known to be involved in these reactions and some systems are almost certainly ubiquitous. This process may occur in a variety of tissues as there is no organ comparable with the liver i.e., the focus for comparable reactions in vertebrates. Activity of the appropriate enzymes often occurs in the midgut, fat body and malpighian tubules. Different species differ widely in their ability to metabolize toxic substances. Amongst plant feeding insects this variation contributes to host pant specificity. The caterpillar of Manduca sexta, for example habitually feeds on alkaloid containing plants, including tobacco and it is able to do his because it detoxifies the alkaloids (Snyder et al., 1994). Although the end products of this metabolic process are commonly excreted, there some times sequestered. Sequestration may also occur without any prior metabolism. In some species and the compound has been stored in the cuticle, perhaps minimizing the risk to the insect. But the other species store the defensive substances in glands or in the haemolymph. Similarly silkworm fat bodies accumulate most of the toxic substances for the detoxification. Greater accumulation of selenium in groups 2, 5, 8 and 11 might have resulted in extensive
degeneration of structure of fat body may be due to the failure of detoxification mechanisms. The most significant changes observed are cell necrosis, fatty degeneration and hyperplasia. Most histological alterations described in fat body of silkworms are available on exposure to pollutants like BHC, malathion and phosphamidon and silkworm exposure to pathogens like Bm NPV and CPV and the changes observed are vacuolization, fatty generation, nuclear degeneration, hyperplasia and cell necrosis (Brown, 1963; Chattoraj and Sharma, 1964; Gawrilescu and Peters, 1931; Hoskins, 1940; Ingram, 1955; Lockau and Ludicke, 1952; McMullen, 1965; Misra 1981). The changes exhibited initially the appearance of destructed membranous of sheath surrounding the fat cells and hypertrophied could be correlated with excessive selenium concentration in haemolymph. Consequently, extravasations of selenium ions diffusing out into surrounding areas might be responsible for excessive damage. Misra 1981, reported that the fat cells are either destroyed or dissolute and scattered nuclei and hypertrophied on exposure to insecticides. Xeros, (1956) reported that the fat body nuclei contained the typical virogenic structure. Jagadish Naik, (2005) reported that 50% tukra fed larvae exhibited mild destructivity and normal vacuolization appeared in the cell of cytoplasm of fat body. The present study has shown that selenium ions exert toxic effect on fat body tissue since soft tissues retain only a small fraction of dietary toxicant (Shupe et al., 1962). It is likely that the structural alterations observed in the present study are consequent of the selenium tissue
retention. Further the alterations observed in the fat body of silkworm are dose dependent and also time dependent. The findings have been further substantiated by biochemical disobsorbents observed in the fat bodies of silkworm treated with different doses of selenium.

The disarranged fat tissue vaculations in fat body cells followed by the shrinkage of fat cells and dissolution of tubular structure suggest that the depletion in its glycogen reserve. Corresponding to the cellular damage to the fat body tissue the decrease in proteins, increase in ammonia, impaired oxidative metabolism reflects the potent toxicity of selenium to fat body.

Even though in the presence of sub lethal dose of selenium is decreased the selenium treated silkworm exhibited some histopathological changes were more at higher selenium dose and at 6 days of exposure. This could be attributed to sodium selenite, which is toxic due to its great solubility and stability. The lower amounts of sodium selenite resulted in very mild histopathological changes in the sub lethal treated silkworm fat body groups 3, 6, 9 and 12. Accumulation and excretion studies have also confirmed the marked decrease in the absorption of selenium in silkworm and less accumulation in the fat body, as well as marked increase of faecal selenium excretion.

In insects both the malpighian tubules and hind gut function together as excretory organs. The malpighian tubules collect the
filtrate from the haemolymph and pass this primary urine to the hind gut. Insect excretion has been reviewed extensively (Springe, 1990, Nicholson, 1993 and Pannabecker, 1995). Since malpighian tubules are mainly concerned with the elimination of undetoxified and unwanted substances from the haemolymph of the silkworm, it is more prone to the toxicity of various toxic pollutants. Selenium treated silkworms in all the groups exhibited significant alterations in the architecture of malpighian tubules. The histopathological changes were in the direct proportion to the dosage and period of selenium administration.

Areas of cloudy swellings, degeneration and necrosis of malpighian tubules could be resulted due to increased selenium ions accumulated (Tables 6 & 7). Similar to the histopathological changes observed in the selenium exposed silkworms in the present investigation are also reported by the earlier workers in different experimental toxicants and insecticides. Misra (1981) reported that those pesticides are excreted through the malpighian tubules. He also pointed out that malpighian tubules are severely damaged in BHC, Malathion and phasmomidon experimental silkworms. He presumed that in the presence of toxic levels of pesticides in the haemolymph the tubular structure of malpighian tubules is selectively damaged by its passage. According to Misra (1981), pesticide exposed malpighian tubules appear degeneration of the cells along with their nuclei, narrowing of the lumen of the tubules and hypertrophied condition.
Histopathological alterations like necrosis of Microvilli, cloudy degeneration of tubular cells in malpighian tubules of poisoned insects have been noticed (Hoskins, 1940; Lockau and Ludicke, 1952; McMullen, 1965). The findings of pioneer investigations showing extensive destruction to the malpighian tubules of selenium intoxicated silkworm, substantiate the observations of the above studies. Higher levels of selenium in the haemolymph and malpighian tubules also support the cytotoxicity to the tubular cells the malpighian tubules.

On the other hand sub lethal dose of selenium also produced certain histopathological changes in 3, 6, 9 and 12 groups of silkworms. The physiological and biochemical alterations are very less and more or less similar to controls in the 9 & 12 groups of silkworms. Correspondingly the structural damage to malpighian tubules in these groups is very less particularly at 6 days of exposure.
Plate I

a, b & c: Transverse sections of the fat body of V instar silkworm *Bombyx mori* L. (PM X NB₄D₂) exposed to lethal and sub lethal doses of selenium at 3 day. X 450 (H&E)

a : Group 1 (Control)
b : Group 2 (Lethal)
c : Group 3 (Sub lethal)
Plate II

a, b & c: Transverse sections of the fat body of V instar silkworm *Bombyx mori* L. (PM X NB₄D₂) exposed to lethal and sub lethal doses of selenium at 4day. X 450 (H&E)

a: Group 4 (Control)
b: Group 5 (Lethal)
c: Group 6 (Sub lethal)
Plate III

a, b & c: Transverse sections of the fat body of V instar silkworm *Bombyx mori* L. (PM X NB₄D₂) exposed to lethal and sub lethal doses of selenium at 5day. X 450 (H&E)

a : Group 7 (Control)
b : Group 8 (Lethal)
c : Group 9 (Sub lethal)
Plate IV

a, b & c: Transverse sections of the fat body of V instar silkworm *Bombyx mori* L. (PM X NB₄D₂) exposed to lethal and sub lethal doses of selenium at 6day. X 450 (H&E)

a : Group 10 (Control)
b : Group 11 (Lethal)
c : Group 12 (Sub lethal)
Plate V

a, b & c: Transverse sections of the Malpighian tubules of V instar silkworm *Bombyx mori* L. (PM X NB4D2) exposed to lethal and sub lethal doses of selenium at 3 day. X 450 (H&E)

a : Group 1 (Control)
b : Group 2 (Lethal)
c : Group 3 (Sub lethal)
Plate VI

a, b & c: Transverse sections of the Malpighian tubules of V instar silkworm *Bombyx mori* L. (PM X NB₄D₂) exposed to lethal and sub lethal doses of selenium at 4day. X 450 (H&E)

a : Group 4 (Control)
b : Group 5 (Lethal)
c : Group 6 (Sub lethal)
Plate VII

a, b & c: Transverse sections of the Malpighian tubules of V instar silkworm Bombyx mori L. (PM X NB₄D₂) exposed to lethal and sub lethal doses of selenium at 5day. X 450 (H&E)

a : Group 7 (Control)
b : Group 8 (Lethal)
c : Group 9 (Sub lethal)
Plate VIII

a, b & c: Transverse sections of the Malpighian tubules of V instar silkworm *Bombyx mori* L. (PM X NB₄D₂) exposed to lethal and sub lethal doses of selenium at 6day. X 450 (H&E)

a : Group 10 (Control)
b : Group 11 (Lethal)
c : Group 12 (Sub lethal)