CHAPTER III

HISTOPATHOLOGICAL
AND HISTOCHEMICAL
OBSERVATIONS
The observations of the present study are discussed here in the following order:

(A) HISTOPATHOLOGICAL STUDY

(i) General histological observations of various organs viz. liver, lung, kidney and intestine of control albino rat.

(ii) Histopathological changes observed in liver, lung, kidney and intestine under following experimental conditions –

a. Acute exposure of cyclophosphamide

b. Acute exposure of plain gluteraldehyde treated erythrocytes

c. Acute exposure of cyclophosphamide loaded gluteraldehyde treated erythrocytes (CPA-GE).

d. Chronic exposure of cyclophosphamide.

(B) HISTOCHEMICAL STUDY

(i) Observations on histochemical change in protein, carbohydrate, nucleic acids and lipid contents of liver, lung, kidney and intestine of albino rats under the following experimental conditions -

a. Acute exposure of cyclophosphamide

b. Chronic exposure of cyclophosphamide.
(i) GENERAL HISTOLOGY

Liver

The liver is the largest gland in the body, which is situated, in the upper and right part of the abdominal cavity. A falsiform ligament divides the liver into two main lobes. The largest part of the liver is covered with peritoneum, which contains a thin capsule of connective tissue. The liver of rat is a dark red gland.

A microscopical examination of the liver reveals that each lobe is composed of small hexagonal lobule enclosed in a connective tissue sheath known as Glisson’s capsule. In the center, is an intralobular vein and radiating out from this are minute vessels called sinusoids which lead intralobular veins in the capsule, which originate from the portal vein. The intralobular veins ultimately join to form the hepatic veins, which take the blood away from the liver. Between the sinusoids, are hepatic cells, which secrete bile. The hepatic cells are polyhedral in shape with spherical nuclei. Bile canaliculi are very fine and difficult to distinguish in routine histological preparations. The connective tissue is present only around the branches of the blood vessels and forms the inner layer of liver capsule (Fig. 3.1).

Liver receives blood from the intestinal tract through the hepatic portal vein and delivers it to the inferior venacava through the hepatic vein. The soluble products of digestion are metabolized in the liver and prepared for assimilation or utilization as fuel elsewhere in the body [Bloom and Fawcett, 1962]. Liver of control rat showed normal histology.
Lungs

The lungs are the essential organs of respiration. They are two in number and located within pleural cavity of thoracic region of the body. Lungs are separated from each other by the heart.

The substance of lung is of a light, porous, spongy texture, it floats in water and it is also highly elastic.

The lungs are composed of a serous coat, a subserous areolar tissue and the pulmonary substance. The serous coat is the pulmonary pleura which is thin, transparent and present inseparably with the lung substance. The subserous areolar tissue, containing large proportion of elastic fibres, invests the entire surface of the lung and extends inwards between the lobules.

The pulmonary substance is composed of lobules, which although closely connected by interlobular areolar tissue, are quite distinct from one another. The lobules vary in size. Each respiratory bronchiole divides into a number of alveolar ducts leading into a number of expanded passage called atria, each of these atria leads into a terminal air sacs called alveoli which is lined by simple flattened epithilium, the cells of which are united at their edges by cement substance. Between these cells polygonal nucleated cells are found (Fig. 3.5).

The respiratory bronchiole and its alveolar duct atria and alveoli related to them constitute a pulmonary unit. These units collectively form the respiratory part of the lung [Davies and Davies, 1962]. Control rats showed normal histology of lungs.
Kidney

The kidney of rat is a dark red, compound tubular gland, which are paired, bean shaped bodies situated in the posterior part of the abdominal cavity. It is composed of an external cortical and internal medullary part.

Histologically, a kidney is composed of large number of tortuous, closely packed tubules, bound together by a connecting stroma. Each tubule consists of two parts, nephron and collecting tubule. The nephron comprises (i) the renal corpuscles which is concerned with the filtration of substances from the plasma; (ii) the renal tubule which is responsible for selective absorption of the substances from the glomerular filtrate. The collecting tubule carries the urine from a number of renal tubules. Renal corpuscles composed of two parts, central glomerulus vessels and the membranous envelope which is termed as glomerular capsule.

The glomerulus is a lobulated tuft of convoluted, capillary blood vessel held together by scanty connective Henle’s loop and distal convoluted tubule which are concerned with the selective absorption of substance from glomerular filtrate. The wall of uriniferous tubule comprises by a simple epithelium with an external covering of basal membrane. Control rats showed normal histology of kidney (Fig. 3.7).

Intestine

The intestine is a tubular and highly coiled structure. The small intestine is divisible into duodenum, jejunum and ileum, which gradually pass one into the next. It is supported from the abdominal wall by the mesentry.

The duodenum is single loop and is the shortest, widest and most fixed part of intestine.
Histologically, wall of the intestine consists of four coats. The outermost is the peritoneal coat then comes the muscular coat, composed of circular and longitudinal muscle layer, the submucous coat composed of areolar tissue and containing blood vessels, nerves follows and the innermost lining is the mucus coat.

The inner surface of the mucus coat is projected into microscopic finger-like processes called villi. Between the villi, are the crypts of Liberkuhn, simple tubular gland. The villi and crypts of Liberkuhn are lined by columnar epithelium, in which mucus secreting goblet cells are scattered and each villi contains a network of blood capillaries and a lymphatic vessel known as lacteal, it also contain muscle fibres.

In the upper part of the submucous coat i.e. in deodenum, are small recemose gland called Brunner's gland and these secrete an alkaline fluid and mucus, their ducts opening either between or into crypts of Liberkuhn (Fig. 3.9).

(ii) HISTOPATHOLOGICAL STUDY

The present study deals with the histopathological changes in liver, lung, kidney and intestine of albino rat under the influence of acute and chronic exposure to cyclophosphamide. The histopathological study has also been performed on acute administration of cyclophosphamide loaded gluteraldehyde treated erythrocytes (CPA-GE).

**Acute study**

Acute study was determined by dividing 32 rats into four groups of 8 rats each. Group I received 0.9% saline solution and acted as a control. Group
II received 25 mg/kg body weight cyclophosphamide (dissolved in 0.9% saline solution) intraperitonially daily up to 96 hours. Albino rats of group III and IV were administered with cyclophosphamide loaded gluteraldehyde treated erythrocytes (equivalent to 6.5 mg/kg body weight) and same amount of plain gluteraldehyde treated erythrocytes respectively through the same route and for the same duration. The rats from different groups were humanely sacrificed simultaneously daily up to 4 days (96 hours). The abdomen of animals were opened and liver, lung, kidney and intestine were excised. After fixation, paraffin blocks were prepared. Sections were cut at 5-6 μm thickness. Sections of different groups were stained for histopathological study and examined under microscope.

Liver

(a) Control rats

The liver of 24, 48, 72 and 96 hours of control rat did not show any marked changes in comparison to that of initial control rats. The liver of control rats comprised of polygonal lobules containing intralobular vein in the center and portal canal at the corners. The bulk of lobules comprising of polyhedral epithelial parenchymal cells (hepatic cells) containing round nuclei has been observed (Fig.3.1).

(b) Administration of free cyclophosphamide

After 24, 48 and 72 hours administration of cyclophosphamide no marked change has been observed but after 96 hours administration of cyclophosphamide, liver of rats showed noticeable histopathological variation in comparison to control rats. Liver of rat showed elongation of hepatic cells.
Hepatic cells seem to be degenerating as evinced by the presence of few necrotic cells (Fig. 3.2).

(c) Administration of plain gluteraldehyde treated erythrocytes

After 24, 48, 72 and 96 hours administration of plain gluteraldehyde treated erythrocytes, histological structure of liver was almost similar to that of control rats (Fig. 3.3).

(d) Administration of cyclophosphamide loaded gluteraldehyde treated erythrocytes (CPA-GE)

At initial, 24 and 48 hours of CPA-GE administration no marked change in liver has been observed as compared to initial control rats but after 72 and 96 hours of exposure of rat to sublethal dose of CPA-GE, few necrotic cells were seen (Fig. 3.4).

Lungs

(a) Control rats

The lungs of 24, 48, 72 and 96 hours of control rat did not show any marked changes in comparison to that of initial control rats (Fig. 3.5).

(b) Administration of free cyclophosphamide

After 96 hours administration of cyclophosphamide, compact alveoli and infiltration of lymphoid tissue on the wall of alveoli have been observed (Fig. 3.6). But no marked change has been observed in 24, 48 and 72 hours.
(c) Administration of plain gluteraldehyde treated erythrocytes

The lungs of rats after 24, 48, 72 and 96 hours administration of plain gluteraldehyde treated erythrocytes showed similar histology as control rats.

(d) Administration of cyclophosphamide loaded gluteraldehyde treated erythrocytes

After 24, 48, 72 and 96 hours exposure of CPA-GE lung showes no marked change in comparison to that of control rats. In contrast to this, great variation was noticed on administration of free cyclophosphamide for the same period.

Kidney

(a) Control rats

The kidney of 24, 48, 72 and 96 hours of control rat exhibited no marked change when compared with the kidney of initial control rats. Kidney of control rats showed a large number of tortuous, closely packed tubules called the uriniferous tubule bound together by a connecting stroma. Circular glomerulus was also seen. Glomerulus contains glomerular capsule (outer envelope) in outside and vessels of glomerulus in inner side of capsule (Fig. 3.7).

(b) Administration of free cyclophosphamide

After 24 and 48 hours of cyclophosphamide administration kidney showed similar structures to that of control rats but after 72 and 96 hours administration degeneration of uriniferous tubule has been observed while glomerulus was unaffected (Fig. 3.8).
(c) Administration of plain gluteraldehyde treated erythrocytes

The kidney of rat showed no marked change from that of control rats after 24, 48, 72 and 96 hours administration of plain gluteraldehyde treated erythrocytes. The histology of kidney was almost similar to that of initial control rats.

(d) Administration of cyclophosphamide loaded gluteraldehyde treated erythrocytes (CPA-GE)

After 24, 48, 72 and 96 hours exposure of CPA-GE lung showed no marked change in comparison to that of control rats. In contrast to this, great variation was noticed on administration of free cyclophosphamide for the same period.

Intestine

(a) Control rats

The intestine of 24, 48, 72 and 96 hours of control rat exhibited similar histology when compared with the intestine of initial control rats (Fig 3.9).

(b) Administration of free cyclophosphamide

After 24, 48, 72, 96 hours administration, of cyclophosphamide intestine showed no remarkable change in comparison to that of control rats.
(c) Administration of plain gluteraldehyde treated erythrocytes

Intestine showed almost similar histological structure to that of control rats after 24, 48, 72 and 96 hours administration of plain gluteraldehyde treated erythrocytes.

(d) Administration of cyclophosphamide loaded gluteraldehyde treated erythrocytes (CPA-GE).

Intestine of treated rats showed no marked change in comparison to that of control rats after 24, 48, 72 and 96 hours administration of CPA-GE.

Chronic study

For chronic study 32 albino rats were divided into 4 groups having 8 rats in each group. Groups I and III were treated as control and administered with 0.9% plain saline solution. Groups II and IV were administered with 10 mg/kg body weight cyclophosphamide (dissolved in 0.9% saline solution) intraperitonially twice a week up to 3 months. The rats comprising from different groups were humanly sacrificed after 30, 60 and 90 days. The abdomen of animals were opened and liver, lung, kidney and intestine were excised and fixed in aqueous Bouin's fluid. After fixation, paraffin blocks of experimental tissue were prepared and sections were cut at 5-6 μm thickness. Sections were stained for histopathological study by haematoxylin and eosin method. Sections were examined under research microscope for histopathological observations.
Liver

(a) Control rats

The liver of 30, 60 and 90 days of control rat exhibited no marked change when compared with the liver of initial control rats (fig-3.1).

(b) Cyclophosphamide treated rats

At initial 30 days administration, no marked change has been observed. However, after 60 and 90 days exposure of cyclophosphamide, large number of necrotic hepatic cells were seen due to fast degeneration of hepatic cells (Fig-3.10). In addition to this parenchymal cells and nuclei were hypertrophied and showed increased volume. Acentric nuclei along with infiltration of lymphoid tissue in hepatic cells have also been observed (Fig-3.11).

Lung

(a) Control rats

The lung of control rats exhibited no marked change in comparison to lung of initial control rats (Fig-3.5).

(b) Cyclophosphamide treated rats

At initial 30 days administration of cyclophosphamide, lung showed similar histological architecture to that of control rats. But after 60 and 90 days administration of cyclophosphamide, lung showed damaged alveolar wall. Damage in the wall of respiratory bronchiole has also been observed (Fig 3.12).
Kidney

(a) Control rats

The kidney of control rats did not show any marked changes in comparison to kidney of initial control rats (Fig 3.7).

(b) Cyclophosphamide treated rats

After 60 and 90 days administration of cyclophosphamide kidney showed noticeable variation in histopathology compared to control rats. Dilatation of uriniferous tubule and vacuolization in tubular epithelium have been observed. Peritoneal covering of the kidney was ruptured at several places due to necrosis in tubular epithelium. The glomerulus appears to be normal (Fig. 3.13).

Intestine

(a) Control rats

The intestine of control rats showed no marked change in comparison to intestine of initial control rats (Fig 3.9).

(b) Cyclophosphamide treated rats

At initial 30 days administration, intestine showed similar histology to that of initial control rats. But after 60 days of administration intestine showed damage in the wall of villi. Fused and elongated Brunner’s gland were also seen during this period (Fig. 3.14). After 90 days administration, ruptured wall of villi and damage were seen in columnar cell lining (Fig. 3.15). In addition to this wall of villi showed vacuolization and damaged wall of Brunner’s gland has been observed (Fig. 3.16).
Fig 3.1. Section of liver from control rat showing polygonal lobules (pl) containing an intralobular vein (iv) in the center. H&E x 100X.

Fig 3.2. Section of liver of rat after 96 hours post treatment with cyclophosphamide. Few necrotic cells (nc) and elongation of hepatic cells (→). H&E x 400 X.
Fig 3.3. Liver of rat after plain gluteraldehyde treatment showing normal histology intralobular vein (iv) in the center and polygonal hepatic cells (hc) at corners. H&E x 100X.

Fig 3.4. Section of liver after 96 hours administration of cyclophosphamide loaded gluteraldehyde treated erythrocytes (CPAGE), showing few necrotic cells (→). H&E x 100X.
Fig 3.5. Section of lung from control rat showing alveoli (AL) and respiratory bronchiole (RBR). H&E x 100X

Fig 3.6. Section of lung of rat after 96 hours administration of cyclophosphamide showing compact alveoli and infiltration of lymphoid tissue in some places (>). H&E x 100X.
Fig. 3.7. Section of kidney from control rat showing normal histology. Glomerulus (Gl) and uriniferous tubules (UT) H&E X 400x.

Fig. 3.8. Section of kidney of rat at 96 hours post treatment with cyclophosphamide. Degeneration of uriniferous tubule (>). H&E X400x.
Fig 3.9. Section of intestine from control rat showing normal histology. Intestinal villi (IV), Brunner's gland (BG). H&E x 100X.
Fig 3.10. Section of liver of rat 90 days post treatment with cyclophosphamide widespread necrosis (→) in hepatic cells. H&E x 100X.

Fig 3.11. Section of liver of rat 90 days post treatment with cyclophosphamide. Hypertrophied parenchymal cells (→) and nuclei with increased volume acentric nuclei. H&E x 400X.
Fig 3.12. Section of lung after 90 days administration of cyclophosphamide showing damaged alveolar wall (→) and damage in the wall of respiratory bronchiole (→). H&E x 100X.

Fig 3.13. Section of kidney of rat at 90 days administration of cyclophosphamide. Dilatation and necrosis in tubular epithelium. (→). H&E X 400x.
Fig 3.14. Section of rat intestine after 60 days administration of cyclophosphamide. Fused and elongated Brunner’s gland. H&E x 400X.

Fig 3.15. Section of rat intestine after 90 days administration of cyclophosphamide showing ruptured wall of villi (→). H&E x 100X.
Fig 3.16. Fig 3.15 in high magnification showing damage in the wall of Brunner’s gland. H&E x 400X.
HISTOCHEMICAL STUDY

The present investigation deals with the histochemical studies in relation to protein, carbohydrate, nucleic acid and lipid distribution in control and at acute and chronic exposure of cyclophosphamide on the liver, lung, kidney and intestine of the albino rat.

Histochemical observations for protein

Acute study

Liver

Liver cells are metabolically very active, having abundant organelles, particularly the mitochondria. Hepatocytes are rich in biochemical contents i.e. glycogen granules, fat globules and vacuoles filled with enzymes.

(a) Control rats

As shown in microphotograph of liver from 24, 48, 72 and 96 hours of control rat (Fig 3.17) the cells were abundant in cytoplasm, granular nuclei. The proteins are uniformly distributed in hepatic cells which are revealed as deep blue colour on treatment with mercuric bromophenol blue stain. The hepatic cells and their nuclei showed high intensity of protein. Bile ductule, bile capillaries and hepatic cords were also intensely protein positive.

(b) Cyclophosphamide treated rats

After 24, 48, 72 and 96 hours administration of cyclophosphamide, protein distribution pattern was similar to that of control rats.
Lung

Lung, the respiratory organ of the mammalian body, not only perform gaseous exchange part of respiration but also produce certain enzymes and protein required for dissociation of carbamino hemoglobin and maintenance of blood pressure of the body through renin-angiotensin-aldosterone system. These activities are closely associated with biochemical pathways of lung.

(a) Control rats

Almost all parts of lung like alveolar wall, alveolar duct, pulmonary artery and blood vessels of 24, 48, 72 and 96 hours of control rat showed rich content of protein which is evinced by deep blue stain due to mercuric bromophenol blue (Fig. 3.19).

(b) Cyclophosphamide treated rats

After 24, 48, 72 and 96 hours administration of cyclophosphamide, lung showed similar protein content to that of control rats.

Kidney

(a) Control rats

The kidney of 24, 48, 72 and 96 hours of control rat showed regular distribution of protein. Proximal and distal uriniferous tubule and collecting ducts were highly protein positive and vessels of glomerulus also showed rich intensity of protein (Fig. 3.21).
(b) Cyclophosphamide treated rats

Kidney of treated rats showed similar distribution pattern of protein as control rats after 24, 48, 72 and 96 hours administration of cyclophosphamide.

Intestine

(a) Control rats

Protein contents were uniformly distributed within the small intestine of 24, 48, 72 and 96 hours of control rat. Serosa, muscular, submucosa and mucosal layer of intestinal wall were richly stained with mercuric bromophenol blue as evinced by deep blue colour which is due to abundant availability of protein in that region. Goblet cells, Brunner's gland, and blood vessels were highly protein positive (Fig. 3.23).

(b) Cyclophosphamide treated rats

Protein distribution pattern remains unchanged after 24, 48, 72 and 96 hours administration of cyclophosphamide in comparison to that of control rats.

Chronic study

Liver

(a) Control rats

The liver of 30, 60 and 90 days of control rat showed similar distribution pattern to that of 96 hours of control rat. Protein contents are uniformly distributed in the sections of liver (Fig.3.17).
(b) Cyclophosphamide treated rats

At initial 30 days administration of cyclophosphamide, liver showed almost similar distribution pattern of protein to that of control rats. But after 60 and 90 days administration, remarkable decrease in protein content has been observed in liver. Hepatic cells showed lower degree of protein intensity in comparison to 96 hours of treated rat. Depletion of protein is severe in some of the hepatic cells. Nuclei, bile capillaries and bile ductule were low protein positive (Fig. 3.18).

Lung

(a) Control rats

Lung of 30, 60 and 90 days of control rat showed similar distribution of protein content as 96 hours of control rat (Fig. 3.19).

(b) Cyclophosphamide treated rats

Similar distribution pattern of protein content has been observed to control rats after 30 days administration of cyclophosphamide. But after 60 and 90 days treatment with cyclophosphamide, lung of rat exhibited decreased protein content on the alveolar wall, alveolar duct and blood vessels when compared to 96 hours of cyclophosphamide treated rats (Fig. 3.20).
Kidney

(a) Control rats

Kidney of 30, 60 and 90 days of control rat showed similar distribution pattern of protein as 96 hours of control rat (Fig. 3.21).

(b) Cyclophosphamide treated rats

At initial 30 days administration of cyclophosphamide, no marked change in protein distribution pattern was observed as compared to control rats. But after 60 and 90 days administration, proximal and distal uriniferous tubule, collecting ducts and vessels of glomerulus were low protein positive as compared to 96 hours of treated rat. Some spaces were formed between uriniferous tubule, due to degeneration and necrosis of tubular epithelium. These spaces were protein negative (Fig. 3.22).

Intestine

(a) Control rats

The small intestine of 30, 60 and 90 days of control rat showed no marked change in protein distribution pattern when compared to initial control rats (Fig. 3.23).

(b) Cyclophosphamide treated rats

No remarkable change has been observed at initial 30 days administration of cyclophosphamide. But after 60 and 90 days administration intestinal villi, goblet cells, Brunner’s gland and blood vessels showed low protein positive in comparison to initial control rats, while serosa and
muscular layers of intestinal wall were highly protein positive due to accumulation of protein in that region (Fig. 3.24).

**Histochemical observations for carbohydrate**

The presence of carbohydrate particularly glycogen was observed by purplish red stain on the treatment with periodic acid Schiff reagent (PAS).

**Acute study**

**Liver**

(a) *Control rats*

In 24, 48, 72 and 96 hours of control liver, glycogen content has been observed to be uniformly distributed throughout the cytoplasm of parenchymal cells (hepatocytes) which are revealed as purplish red color on treatment with PAS stain. Glycogen content was more marked in and around blood vessels and around the intralobular vein while bile canaliculi and bile ductule were free from glycogen which is evinced by negative staining of glycogen with PAS stain (Fig. 3.25).

(b) *Cyclophosphamide treated rats*

After 24, 48, 72 and 96 hours exposure of cyclophosphamide liver showed no marked change in distribution pattern of glycogen contents as compared to control rats.

**Lung**

(a) *Control rats*

The lung of 24, 48, 72 and 96 hours of control rat showed intensely PAS positive stain in respiratory bronchiole, pulmonary artery, alveoli and blood
capillaries, which is rich in glycogen contents. The distribution of glycogen was almost uniform in all parts (Fig 3.27).

(b) Cyclophosphamide treated rats

After 24, 48, 72 and 96 hours administration of cyclophosphamide lung showed almost similar distribution pattern of glycogen content as to control rats.

Kidney

(a) Control rats

The kidney of 24, 48, 72 and 96 hours of control rat showed PAS positive glomerulus proximal and distal uriniferous tubule. Glycogen deposition was more marked in proximal and distal uriniferous tubule (Fig. 3.28).

(b) Cyclophosphamide treated rats

After 24, 48, 72 and 96 hours post treatment with cyclophosphamide, the distribution pattern of glycogen contents remains unchanged, when compared with control rats.

Intestine

(a) Control rats

The small intestine of 24, 48, 72 and 96 hours of control rat showed intensely PAS positive nuclei in the wall of Brunner’s gland and mucosa. Fine network of blood vessels was highly PAS positive. Blood capillaries and connective tissues, present in muscle layer, submucosa and mucosa layer of intestine were moderately PAS positive (Fig. 3.29).
(b) Cyclophosphamide treated rats

Glycogen contents were similar to that of control rats after 24, 48, 72 and 96 hours administration of cyclophosphamide.

Chronic study

Liver

(a) Control rats

The liver of 30, 60 and 90 days of control rat showed similar distribution pattern of glycogen to that of initial (96 hours) control rat (Fig. 3.25).

(b) Cyclophosphamide treated rats

During initial stages of cyclophosphamide treatment (30 days), no marked change in glycogen contents have been observed in liver. But after 60 to 90 days administration, heavy accumulation of glycogen has been observed in parenchymal cells or hepatocytes which was apparently observed as qualitative increase in glycogen content in hepatic cells in contrast to control rats (Fig. 3.26).

Lung

(a) Control rats

Lung of 30, 60 and 90 days of control showed similar distribution pattern of glycogen to that of initial control rats (Fig. 3.27).
(b) Cyclophosphamide treated rats

After 30, 60 and 90 days administration of cyclophosphamide, lung showed no marked change in glycogen distribution pattern in comparison to initial control rats.

Kidney

(a) Control rats

The kidney of 30, 60 and 90 days of control rat showed no marked change in distribution pattern of glycogen when compared with the kidney of 96 hours of control rats (Fig. 3.28).

(b) Cyclophosphamide treated rats

Kidney of rat showed similar distribution pattern of glycogen as control rats after 30, 60 and 90 days administration of cyclophosphamide.

Intestine

(a) Control rats

Small intestine of 30, 60 and 90 days of control rat showed no remarkable change in glycogen distribution in comparison to 96 hours of control rat (Fig. 3.29).

(b) Cyclophosphamide treated rats

Small intestine after 30, 60 and 90 days administration showed similar distribution pattern of glycogen as to initial control rats.
Histochemical observations for nucleic acid

In general, the nuclei and nucleic acid (DNA and RNA) stained green but in some cases the nuclei turned dark reddish-green which may be due to pH changes because it is reported that at lower pH the pyronin components stained more strongly while at higher pH 4.5 to 5 the effect of methyl-green is accentuated [Pearse, 1960].

Acute study

Liver

(a) Control rats

Liver from 24, 48, 72 and 96 hours of control rat showed uniform distribution of nucleic acids (DNA and RNA) in hepatic cells. The nuclei were intensely DNA and RNA positive. RNA is abundantly distributed throughout the cytoplasm of hepatic cells as evinced by rich staining of methyl green pyronin Y (Fig. 3.30).

(b) Cyclophosphamide treated rats

In 24, 48, 72 and 96 hours of cyclophosphamide treated rat, nucleic acid content of liver has been observed almost similar to that of control rats.

Lung

(a) Control rats

The lung of 24, 48 72 and 96 hours of control rat showed symmetrically DNA positive nuclei. Uniform distribution of cytoplasmic RNA was seen around the wall of alveoli, blood vessels, pulmonary vein and respiratory bronchiole having dense chromatin(Fig 3.32).
(b) Cyclophosphamide treated rats

After 24, 48, 72 and 96 hours of cyclophosphamide administration, no remarkable change in nucleic acid content has been observed when compared to lung of control rats.

Kidney

(a) Control rats

Kidney of 24, 48, 72 and 96 hours of control rat showed rich staining methyl-green pyronin Y in nuclei, which showed abundant availability of DNA in nucleus. RNA contents have been uniformly distributed throughout the cytoplasm of cells of uriniferous tubule (both proximal and distal) and collecting duct. Glomerulus. Showed intensely DNA positive nuclei (Fig. 3.34).

(b) Cyclophosphamide treated rats

Kidney, after 24, 48, 72 and 96 hours of cyclophosphamide administration, did not show any change in distribution pattern of nucleic acids in comparison to control rats.

Intestine

(a) Control rats

The small intestine of 24, 48, 72 and 96 hours of control rat showed DNA positive nuclei in goblet cells, present in intestinal villi. DNA and RNA positive nuclei have also been observed in Brunner’s gland and throughout the cytoplasmic matrix. Cytoplasmic RNA is abundantly distributed in the cytoplasm of cell of intestinal villi, Brunner’s gland, blood vessels and blood
capillaries. RNA positive materials are more pronounced in intestinal villi than the other parts (Fig. 3.36).

(b) Cyclophosphamide treated rats

After acute exposure of cyclophosphamide small intestine showed similar distribution pattern of nucleic acid as control rats.

Chronic study

Liver

(a) Control rats

The liver of 30, 60 and 90 days of control rat showed similar distribution pattern of nucleic acid content in liver as compared to 96 hours of control rat (Fig. 3.30).

(b) Cyclophosphamide treated rats

There has been no remarkable change in nucleic acid distribution pattern in liver during 30 and 60 days administration of cyclophosphamide, in comparison to initial control rats. However after 90 days administration, the nuclei showed feeble staining with methyl-green pyronin Y, which showed decreased DNA and RNA content in nuclei. Cytoplasm of hepatic cells, bile canaliculi and bile duct also showed decreased RNA content which was exhibited by less staining of cytoplasmic RNA with methyl-green pyronin Y in that region (Fig. 3.31).
Lung

(a) Control rats

Lung of 30, 60 and 90 days of control rat showed similar distribution pattern of nucleic acid in respiratory bronchiole, wall of alveoli, pulmonary vein and throughout the cytoplasm as to initial (96 hours) control rat (Fig. 3.32).

(b) Cyclophosphamide treated rats

Lung showed no marked change in nucleic acid distribution pattern upto 60 days in comparison to control rat. But after 90 days administration, lung exhibited decreased RNA content throughout the cytoplasm. Nuclei were DNA positive but they were few in numbers. Cytoplasm of respiratory bronchiole showed less staining with methyl-green pyronin Y which showed decreased RNA content in the cytoplasm (Fig. 3.33).

Kidney

(a) Control rats

Kidney of 30, 60 and 90 days of control rat showed no noticeable change in nucleic acid contents. Kidney showed similar distribution pattern of nucleic acid as initial control rats (Fig. 3.34).

(b) Cyclophosphamide treated rats

For initial 30 and 60 days administration of cyclophosphamide no appreciable change in nucleic acid content has been observed when compared to control rats. After 90 days of drug treatment kidney showed diminished RNA content in the cytoplasmic matrix. The depletion was more pronounced
in the cytoplasm of uriniferous tubules. Slight decrease in DNA positive nuclei have also been observed (Fig. 3.35).

**Intestine**

(a) Control rats

The small intestine of 30, 60 and 90 days of control rat showed similar distribution pattern of nucleic acid in cytoplasmic matrix of intestinal villi, goblet cells, Brunner’s gland as 96 hours of control rat (Fig 3.36).

(b) Cyclophosphamide treated rats

After 90 days administration of cyclophosphamide small intestine showed decrease in DNA positive nuclei in goblet cells in comparison to 96 hours of control rats. Brunner’s gland did not show any change in DNA content. All parts of intestine like serosa, circular muscle layer, longitudinal muscle layer, submucosa and mucosa layer showed depletion of RNA positive material in their cytoplasm (Fig. 3.37). However after 30 to 60 days administration of cyclophosphamide nucleic acid (DNA and RNA) contents were similar to that of control rats.

**Histochemical observations for lipids**

Lipid took black stain on the treatment with sudan black ‘B’.

**Acute study**

**Liver**

(a) Control rats

In 24, 48, 72 and 96 hours of control liver, lipids appeared as small droplets in the cytoplasm of hepatic cells. Little patchy deposition of lipids was seen in some of the hepatic cells (Fig. 3.38).
(b) Cyclophosphamide treated rats

After 24, 48, 72 and 96 hours of cyclophosphamide treated rat showed no marked change in lipid content in liver when compared to liver of control rats.

Lung

(a) Control rats

Sudanophilic lipids are shown to be uniformly distributed throughout the cytoplasm of lung of 24, 48, 72 and 96 hours of control rat (Fig. 3.40).

(b) Cyclophosphamide treated rats

After 24, 48, 72 and 96 hours administration of cyclophosphamide, lung showed similar distribution of lipid to that of control rats.

Kidney

(a) Control rats

The kidney of 24, 48, 72 and 96 hours of control rat showed uniform distribution of sudanophilic lipids around the wall of proximal and distal uriniferous tubule while connective tissues showed negative staining with Sudan Black B (Fig. 3.42).

(b) Cyclophosphamide treated rats

In the present study, after 24, 48, 72 and 96 hours of cyclophosphamide administration, sections of kidney showed no remarkable change in lipid distribution pattern in comparison to control rats.
Intestine

(a) Control rats

Lipids were found uniformly distributed in cytoplasmic matrix, blood capillaries, and blood vessels in 24, 48, 72 and 96 hours of control rat. Lipid deposition was seen in intestinal villi particularly lacteals (Fig. 3.44). The serosa, muscle layer, submucosa and mucosa layer of the intestinal wall was also showed uniform distribution of lipids (Fig 3.45).

(b) Cyclophosphamide treated rats

Lipid distribution pattern remains unchanged in small intestine after 24, 48, 72 and 96 hours exposure of cyclophosphamide, as compared to control rats.

Chronic study

Liver

(a) Control rats

In 30, 60 and 90 days of control liver, lipid contents were similar to that of 96 hours of control rat (Fig 3.38).

(b) Cyclophosphamide treated rats

During initial stages of cyclophosphamide treatment (30 and 60 days), no marked change in lipid distribution pattern has been observed as compared to 96 hours of treated rat. But after 90 days administration, patchy deposition of lipids in many of the hepatic cells were seen. The deposition of lipids was more marked around the intralobular vein which was apparently observed as qualitative increase in lipid content (Fig.3.39).
Lung

(a) Control rats

The lung of 30, 60 and 90 days of control rat showed similar lipid distribution pattern as 96 hours of control rat. (Fig 3.40).

(b) Cyclophosphamide treated rats

After 90 days administration of cyclophosphamide heavy lipid infiltration has been observed around the wall of alveoli, which was apparently observed as increase in lipid content in that region in comparison to control rats. The wall of pulmonary artery, pulmonary vein and respiratory bronchiole showed more deposition of lipids (Fig. 3.41). However for initial days of administration (30 and 60 days) lipid content remains unchanged in comparison to control rats.

Kidney

(a) Control rats

Kidney of 30, 60 and 90 days of control rat showed similar distribution of lipid to that of 96 hours of control rat (Fig 3.42).

(b) Cyclophosphamide treated rats

In the present investigation after 30 and 60 days administration of cyclophosphamide, kidney showed no change in lipid content in comparison to the kidney of control rats. But after 90 days exposure patchy deposition of lipids was seen in glomerular region and wall of some of the uriniferous tubules (Fig. 3.43).
Intestine

(a) Control rats

Small intestine of control rat showed similar lipid distribution pattern as 96 hours of control rat (Fig. 3.44).

(b) Cyclophosphamide treated rats

At initial stages (30 and 60 days) of cyclophosphamide treatment, distribution pattern of sudanophilic lipids remains unchanged in comparison to control rats. However, slight increase in lipid content was seen in serosa, circular and longitudinal muscle layer of intestinal wall, as compared to control rats (Fig. 3.46). Increased lipid contents were also observed in blood vessels, connective tissues and intestinal villi (Fig. 3.47).
Fig 3.17 Liver from control rat showing uniform distribution of protein (P) (deep blue stain) in hepatic cells, nuclei, bile capillaries and bile duct. Mercuric bromophenol blue stain x 400X.

Fig 3.18 Section of liver after 90 days administration of cyclophosphamide showing decrease in protein content in hepatic cells. The depletion being severe in some of the hepatic cells Mercuric-bromophenol blue x 400X.
Fig 3.19 Section of lung from control rat showing uniform distribution of protein (P) in alveolar wall, alveolar duct and blood vessels. Mercuric Bromophenol blue x 400X.

Fig 3.20 Section of rat lung after 90 days administration of cyclophosphamide showing decreased protein content in alveolar wall and blood vessels (→). Mercuric Bromophenol blue stain x 400X.
Fig 3.21 Section of kidney of control rat showing uniform distribution of protein in uriniferous tubule, glomerulus and collecting tubule. Mercuric bromophenol blue stain x 100X.

Fig 3.22 Section of kidney after 90 days administration. Low protein intensity in uriniferous tubule (VT), glomerulus (Gl) and collecting tubule (CT). Mercuric bromophenol blue stain x 100X.
Fig 3.23 Section of small intestine of control rat showing uniform distribution of protein (P) in intestinal wall, Brunner's gland, intestinal villi and blood vessels. Mercuric-Bromophenol Blue x 100X.

Fig 3.24 Section of small intestine after 90 days administration of cyclophosphamide. Goblet cells, Brunner's gland, blood vessels are low protein (P) positive. Accumulation of protein in the Serosa, transverse and longitudinal muscle layer. Mercuric Bromophenol Blue stain x 100X.
Fig 3.25 Section of liver of control rat showing uniform distribution of glycogen in hepatic cells. Glycogen content (Purplish red stain) is more marked around the intralobular vein and blood capillaries. PAS x 400X.

Fig 3.26 Section of liver after 90 days administration of cyclophosphamide showing substantial increase in glycogen content in hepatic cells (→) PAS x 400X.
Fig 3.27 Section of lung of control rat showing intensely PAS positive respiratory bronchiole (RB), alveolar wall (AL), Blood vessels (BV), and cytoplasmic matrix. PAS x 100X.

Fig 3.28 Kidney from control rat. Intensely PAS positive proximal and distal uniferous tubule (UT) and glomerulus (GL). PAX x 100X.
Fig 3.29 Intestine from control rat showing PAS positive nuclei in goblet cells (GB) of Brunner's gland (BG) PAS x 400X.
Fig 3.30 Liver from control rat showing intensely DNA positive nuclei (N) and RNA positive cytoplasm in hepatic cells (HC). Methyl-green pyronin Y x 400X.

Fig 3.31 Liver after 90 days treatment with cyclophosphamide showing decreased DNA and RNA content in nuclei (→) and depletion of RNA content in the cytoplasm of hepatic cells (★). Methyl-green pyronin Y x 400X.
Fig 3.32 Lung from control rat showing symmetrically DNA positive nuclei (➔).
Uniform distribution of cytoplasmic RNA (green stained) are seen around the
wall of alveoli (AL), Blood vessels (BV) and Respiratory bronchiol(RS),
methyl-green pyronin Y x 100X.

Fig 3.33 Lung after 90 days administration of cyclophosphamide showing
decrease in cytoplasmic RNA (➔). Few DNA positive nuclei are present.
Methyl-green pyronin Y stain x 100X.
Fig 3.34 Kidney from control rat showing uniform distribution of Nucleic acid (NA) (DNA and RNA) in nucleus and RNA in cytoplasm. Methyl-green pyronin Y stain x 100X.

Fig 3.35 Kidney after 90 days administration of cyclophosphamide showing decreased RNA content in the cytoplasm of uriniferous tubule and few DNA positive nuclei. Methyl-green pyronin Y stain x 100X.
Fig 3.36 Intestine from control rat. Intensely DNA and RNA positive nuclei (N) (dark stained) are present and abundant distribution of cytoplasmic RNA in intestinal villi, Brunner's gland and throughout the cytoplasm. Methylgreen pyronin Y Stain x 100X.

Fig 3.37 Intestine after 90 days post treatment with cyclophosphamide showing decreased RNA content in the cytoplasmic matrix. Methyl green pyronin Y x 100X.
Fig 3.38 Liver from control rat showing small droplets of lipid (L) in hepatic cells. Little patchy deposition of lipids are seen in some of the hepatic cells (†). Sudan Black B stain x 100X.

Fig 3.39 Liver after 90 days post treatment with cyclophosphamide showing patchy deposition of lipid in many of the hepatic cells (†). Sudan Black B stain x 100X.
Fig 3.40 Lung from control rat showing uniform distribution of lipid (L) in the cytoplasm. Sudan Black B x 100X.

Fig 3.41 Lung after 90 days administration of cyclophosphamide showing increase in lipid content due to heavy lipid deposition on the wall of alveoli (arrow). Sudan black B stain x 100X.
Fig 3.42 Kidney of control rat showing uniform distribution of lipids in uriniferous tubule. Sudan Black B stain x 100X.

Fig 3.43 Section of kidney of rat after 90 days administration of cyclophosphamide. Patchy deposition of lipids are seen (→). Sudan Black B stain x 100X.
Fig 3.44 Section of small intestine of control rat showing uniform distribution of lipids in muscle layer and submucosa layer. Lipid deposition are seen in lacteals. Sudan Black B x 100X.

Fig. 3.45. Fig 3.44 in high magnification. Small intestine showing uniform distribution of lipids in muscle layer and submucosa layer (→). Sudan black B X 400x.
Fig 3.46 Small intestine of rat after 90 days administration of cyclophosphamide showing increased lipid content in muscle layer (→). Sudan black B X 450x.

Fig 3.47 Section of small intestine after 90 days administration of cyclophosphamide showing increased lipid contents in intestinal villi. Sudan black B x 100X.