CHAPTER II

PERTINENT LITERATURE
This review of literature chiefly deals with the pertinent information regarding the toxic effects of manganese and other metals on the urinary system and testicular tissue in man and animals. Particular emphasis has been given to the histoenzymic and histological changes in kidney and testis by metals like manganese, cadmium, mercury and lead. The mechanism of action of various metals and mechanism of testis calcification has been briefly highlighted.

**Manganese requirements in rats and rabbits**

The normal values of dietary requirement of manganese in rabbits and rats are important in view of the toxicity studies conducted on manganese in these animals. The work of Smith and Ellis (1947) indicate that rabbit requires about 300 μg of inorganic manganese per day for satisfactory growth. Similarly normal requirement for rat is 0.2 mg per day (Holt Kamp and Hill, 1950). The excess of manganese if given to these animals, however produces toxic effects on body tissues mainly brain, liver, kidney and testis.

**Urinary System**

The normal concentration of manganese in kidneys of rabbits and rats was 0.087 to 0.101 milligram per 100 grams wet weight (Bertrand and Medigreceanu, 1913).
According to Lund, Shaw and Drinker (1921) the normal concentration of manganese in the kidney tissue was 0.147 (rabbit) and 0.110 (rat) milligram per 100 grams wet weight.

The normal excretion of manganese through kidney is negligible. Handowsky, et al. (1923) after giving subcutaneous injections of various salts of manganese found that manganese was mainly excreted in feces whereas only traces were found in urine. According to Thomas (1970) only 0.1 to 3% of normally absorbed manganese was excreted in urine. It is known that excessive exposure to manganese results in higher urinary manganese concentrations (Tolonen, 1972). After administration of radioactive manganese, it rapidly disappears from the circulation and is stored in organs rich in mitochondria such as liver, kidney and pancreas (Cotzias, 1958). Kobert (1981), Cahn (1984) and Harnack and Schreiber (1901) found highest manganese concentrations in kidneys after giving manganese salts to animals.

There are many reports of renal damage in animals treated with manganese. Renal damage has also been reported with other metals like cadmium, mercury and lead. Kobert (1983) noted nephritis in some of the animals which had received subcutaneous injections of manganese citrate. Findlays (1924) found albumen, red blood cells, casts and bile pigments in the urine of manganese treated animals. The glomeruli were found to be congested and some of the
tubules showed fatty degeneration. Handowsky et al. (1925) Grueinstein and Popowa (1928) have reported degenerative changes in kidneys of animals which were intoxicated with manganese compounds.

Hurst and Hurst (1928) studied changes in the renal tissue of guinea pigs and rabbits and described rabbits being more susceptible to the action of manganese than guinea pigs. In guinea pigs the cells of convoluted tubules and Henle's loop were degenerated. Fatty degeneration in the cells of descending limbs of Henle's loop and convoluted tubules was also observed. In rabbits focal areas of cortical necrosis, with wide spread haemorrhages, foci of polymorphonuclear infiltration and fatty degeneration have been reported. The glomeruli were congested. At places complete obliteration of glomerulus with necrosis was seen. In the loops of Henle cell casts were found in the lumen. The histoenzymic studies on renal tissue in chronic manganese toxicity has been undertaken by Jonek et al. (1965). They have reported reduction in respiratory ferment and ATP. The fall in ATP has been suggested to lead to the retention of Na⁺ and K⁺ in the kidney. An increased acid phosphatase activity has been suggested to have lead to cause degeneration of the tubules.

Certain other metals are known to produce renal damage. In cadmium poisoning the renal damage has been reported by Badder (1951) and Bonnell (1955) in the form of diffused interstitial fibrosis with degenerative changes in many glomeruli and tubules. Highest concentration
of mercury has been found in kidneys after mercury administration to animals (Browning, 1969). The accumulation of mercury chiefly takes place in the cortical and subcortical regions of the kidney (Hergstrand et al., 1958). The toxic effect of mercury was mainly found to be on the tubular epithelium producing albumen and cell casts in the urine. In a few cases of mercury poisoning toxic nephrosis has been reported. Proteinuria is the main finding in acute and subacute poisoning (Zangger, 1930).

In lead poisoning degenerative changes in the renal tubules and vascular lesions in the form of multiple haemorrhages and thickening of the wall of blood vessel thus leading to nephrosclerosis have been reported (Calvery, 1938; Fishberg, 1939; Fairhall and Miller, 1941). In clinical cases of lead poisoning progressive renal damage and interstitial nephritis has been observed (Lane, 1949; Oliver, 1911).

The studies so far conducted are unable to explain the initial site of action of manganese since histoenzymic studies at a stage earlier than histological changes in this tissue have not been conducted so far. Further the effects of manganese on ureter and urinary bladder have not been investigated as evident from the available literature. The present study has been conducted with the above viewpoint to study detailed renal pathology and early histoenzymic changes in kidney in manganese toxicity.
Testis:

Male rats or rabbits fed on manganese deficient diet are known to have retarded growth and also to develop testicular degeneration, lack of sexual interest and sterility (Orent and McCollum, 1931; Boyer et al., 1942; Smith et al., 1944). The poor growth and sterility are, however, reversible by the administration of manganese (Shils and McCollum, 1943). The fact that animals getting insufficient dietary manganese develop arrested spermatogenesis (Orent and McCollum, 1931) it was suspected that manganese like zinc, might be incorporated into some phases of development of the spermatozoa. Radioisotope of manganese has demonstrated that manganese taken up by testis was incorporated into the products of testis, which were transported into the epididymis (Gun et al., 1970). Some radioactive manganese was also found associated with interstitial tissue.

In view of the possible role suspected for manganese in the reproductive system of the male, the knowledge about its physiologic significance in the testis is not elaborate (Johnson et al., 1970).

However, in manganese poisoning male sexual impotence is one of the most common manifestations of manganese toxicity (Penalver, 1955), which occurs fairly early in the disease process (Balani et al., 1967). Impotence follows a period of sexual stimulation and
dimunition of libido (Penalver, 1955; Rodier, 1955 and Schuler et al., 1957). However, there is not a single report on the toxicity of manganese in testis of clinical cases of manganese poisoning virtually on account of no efforts by investigators to investigate the possible injury in this organ.

The first study on the toxic effects of manganese on rats testis was reported by Chandra in 1971. Sperm producing cells were found to be depleted in degenerated tubules with multinucleated cells formation in these tubules. In later study by Kar et al. (1972) the manganese concentration in rat testis showed a 4- to 9-fold increase at 30 and 60 days after manganese administration respectively. Besides, a significant rise in certain amino acids i.e. alanine, cysteine, leucine, proline, phenylalanine and glutamate was also found at 60 days. These workers concluded that manganese might have an inhibitory effect on various enzymes thus leading to accumulation of certain amino acids which were otherwise utilized.

Chandra et al. (1973) reported that manganese treated rabbits (I/T MnO₂) proved sterile, when kept with females of proven fertility. They reported in testis, histological changes in the tubular epithelium in the form of degeneration and patchy calcification of tubules. The biochemical changes observed were decrease in SDH, ATPase and acid phosphatase activities at 240 days.
it was suggested that manganese by producing 
activity affected the energy metabolism and produced the 
degeneration of sperm producing structures. Decrease in 
APase and acid phosphatase activities was suggested to 
be due to either slow metabolism or for the preservation 
of phosphate esters to meet the energy supply of tissue.

Seth et al. (1973) in an effort to understand the 
mechanism of injury studied biochemical changes and 
histopathological alterations at 2, 4, 6 and 8 months 
after single intratracheal administration of manganese. 
The APase activity was found to be decreased at 2 months 
at which the histological damage was also present in 10 
to 20% of the seminiferous tubules. The decrease in 
these enzymes and testicular degeneration were more 
marked with lapse of time.

The above studies, however, do not explain the 
initial site of action of manganese and also the 
biochemical alterations preceding the histopathological 
changes. The enzyme alterations observed were too late 
from the viewpoint of establishing the original site of 
action and sequence of biochemical events that might 
have taken place in testis under the toxic influence of 
manganese.

The toxic effects of metals like aluminium, 
copper, iron, lead, mercury and nickel etc. on the 
testis of rats and mice has been reported by Kamboj and
Kar, 1964. The necrosis of testicular tissue was produced after direct intratesticular injection of the metal although the damage produced by direct injections in testis is nonspecific (Johnson et al., 1970). Mercury which is a nonessential element does not cause testicular necrosis even after injection of lethal dose (Johnson et al., 1970). Timm and Schulz (1966) found in the Leydig cells, granules after intraperitoneal injections of mercury salt, which were attributed to the presence of mercury deposits in testis. Lead is also injurious to testis (Hamilton and Hardy, 1949) as it has deleterious effects on male germ cells. Cadmium is highly injurious to testis and it seems to have selective action on testicular tissue, since a dose as low as 0.01 to 0.02 mole/kg produces degenerative changes in testis (Johnson et al., 1970). The evidence at hand indicates conclusively that in cadmium toxicity the spermatogenic epithelium is not the original site of injury. It produces typical hemorrhagic necrosis in testis. The histoenzymic studies in testis after cadmium administration have shown increase in alkaline phosphatase in the capillary endothelium (Nae Kawa et al., 1963) and decrease in SDH, LDH and NADPH, which are primarily associated with seminiferous tubules (Dimow and Knorre, 1967). However, these changes may not be due to direct effect of cadmium on epithelium. It is considered a secondary reaction from loss of nutritives following interference with the blood supply.
Besides a large number of metallic salts including manganese are known to produce calcification of testis (Sharma, 1972; Chandra, 1973).

**Mechanism of calcification in the testicular tissue**

Sporadic calcification of the seminiferous tubules of various animals has been reported in 2-30% cases by several investigators (Albrecht, 1932; Webster, 1932; Barker, 1948, 1956 and Fraser and Wilson, 1966). In such cases tubular degeneration and intratesticular calcification is commonly seen which is considered to be secondary to degenerative changes. But this does not necessarily indicate infertility as highly fertile bulls are observed with some areas of testicular calcification (Barker, 1956). In goats the incidence of testicular calcification is observed in 30-40% which usually lead to sterility (Fraser and Wilson, 1966).

The skeleton acts as the reservoir for calcium and plays important role in hypercalcaemia and in the pathological calcification.

The calcium level in the blood depends on several factors such as calcium intake, serum phosphorus levels vitamin D and a number of hormones. The interplay of these factors is responsible for maintaining a dynamic equilibrium of calcium between blood, bone and soft tissues.
Vitamin D and the parathyroid hormone (PTH) have a vital role in maintaining Ca\textsuperscript{++} and HPO\textsubscript{4}\textsuperscript{-} level in a state of equilibrium which would not exceed the level at which soft tissues become susceptible to its precipitation (Neuman and Neuman, 1953). The disturbance in the ionic equilibrium of Ca\textsuperscript{++} and HPO\textsubscript{4}\textsuperscript{-} may lead to either systemic or local calcification. Degenerating and necrotic foci in soft tissues are calcified in due course of time. This is called as "dystrophic calcification".

In vitro studies by Henrichsen (1959) have emphasized that the calcification does not occur in cells until they are necrosed. The necrosed cells liberate alkaline phosphatase which help in apatite crystal formation. Selye (1962) mentioned that there is absolutely no relationship between degeneration and necrosis of a cell and deposition of calcium.

The role of alkaline phosphatase in calcium phosphate deposition in tissue, discussed by Gomori (1943), Axelrod (1949), Meyerhof and Green (1949), Trout (1959) and Grant et al. (1963) suggest that the state of tissue itself is primarily responsible for calcium deposition and it is not dependent on the level of serum calcium. Thomas et al. (1956) suggested that once deposition of mineral apatite is initiated during calcification further calcification may occur in even low concentrations of Ca\textsuperscript{++} and HPO\textsubscript{4}\textsuperscript{-} than that required for initiation of the process.
At present it is generally held that collagenous fibres play the unique property of a seeding mechanism which initiates crystallization of calcium phosphate in a regular manner along the fibres (Neuman and Neuman, 1958; Glimcher, 1959; Santanam, 1959; Namachandran and Santanam, 1959; Fleisch and Neuman, 1960; Boros et al., 1967; Wadkins, 1968; Gabbiani et al., 1970).

**Mechanism of action of metals**

Many of the essential trace metals (Weinberg, 1962) produce markedly deleterious effects when administered or absorbed in amounts in excess of normal requirements (Bittar and Bittar, 1969). Excess of metal ions have been shown to exert their toxic effects by affecting cellular physiology in several ways (Passow et al., 1961). The amount of metal ions required to produce an injurious response varies from metal to metal illustrating the diversity of mechanism involved in their toxicity (Bittar and Bittar, 1969). The metals possessing a density greater than five, often referred as heavy metals are specially toxic to the living system.

It is, therefore, very difficult to give generalization of the pharmacological action of metals since they do not have common chemical properties. It has been shown that metal ions have affinity for a wide variety of organic molecules of biological significance such as lipids, amino acids, co-enzymes, enzymes and other proteins containing sulphur, nitrogen or oxygen.
which act as electron donors to form complexes with the metals. Besides, acting on the enzyme molecule itself metal ions have been shown to react with the substrate, cofactors, and activators. In living system at least following ligands can be expected to be present

-\( \text{-OH} \), -\( \text{COOH} \), -\( \text{PO}_3\text{H}_2 \), -\( \text{SH} \), \( \text{NH}_2 \), -imidazole. These ligands form integral part of the biomolecules, essential for the normal functioning of the cells. The site of metal action in the cell can be predicted on account of the knowledge about the relative affinity of metal for a particular type of ligand.

The metal ions interfere with the action of enzyme and other functional proteins. The enzyme inhibition results in the increase in substrate concentration, thereby resulting in altered physiological activity of the cell. The whole enzyme system including the enzyme, substrate, cofactors and activators, may be involved by binding of any single component with the metal. The majority of enzymes especially those having \( \text{SH} \)-groups can be inhibited by metals. Certain metals are known to stimulate cellular activity in low concentrations below inhibitory level and this phenomenon is referred as Schultz Arndt Rule (Clark, 1937).

The action of metal ions also depend on biological factors, the chemical composition and the structural as well as the functional organization of cells (Passow et al. 1961). The cellular structure greatly influences the
accessibility of the metal to sensitive ligands. The sequence of events are from the outside of the cell towards the inside. The binding of metal ions with the ligands of the cell surface disturbs the membrane function such as membrane permeability and transport (Linderholm, 1952; Ussing and Zerahn, 1951). The metal binding with ligands takes place at the sensitive or insensitive sites. Thus the toxic effects of metal may be produced only by a very small part of the total metal which binds at the sensitive site in the cell; in other words binding with insensitive sites protects the cell to some extent against the toxic action of the metal. Since metal can disturb any existing biological activity, theoretically as many as responses can be found as there are activities. Based on the factors of anatomical structure and the nature and accessibility of biological components certain types of responses predominate.

The distinction between primary and secondary effects is important. The primary response is the reduction in the activity of the site with which the metal binds. The secondary response is the reduction in the activity of another system dependent in some manner on the binding site.

In attempting to trace the mechanism of action it is most important to study the primary rather than the end results of metal toxicity, since the inactivation of one sensitive site by a metal may induce secondary
changes which ultimately affect the cell physiology.

In intact animals at organ level the action of metals follows some generalizations as in the intact cell. For each metal a particular organ may be more susceptible than other organs. However, here within organ several cell populations of different susceptibilities may exist in a complex anatomical arrangement. This is due to inherent susceptibilities of certain cells or higher concentrations of metal deposited in these cells (Passow et al., 1961). The susceptibility of the renal tubular cells to mercury and uranium has been attributed to local factors which lead to a high rate of deposition of the metal.

In animals factors like absorption, distribution, deposition and excretion govern the effective concentration and exposure time required for cell damage. The physiological response after toxicity, is determined by the biological factors viz. chemical, structural and functional organization of the organ (Passow et al., 1961).