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Morphological Changes in Liver and Kidney of the Common Ground Squirrel, *Funambullus pennant* Following Acute Cadmium Exposure

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ABSTRACT A single dose of cadmium acetate by intraperitoneal injection produced several histologic alterations in the liver and kidney of the common Indian ground squirrel (*Funambulus pennanti*, Wroughton 1905). Within 7 days after Cd exposure, the test animals revealed an increase in liver and kidney weights ($P<0.05$). The liver disorder resulted in dilatation of the central vein, Kupffer cells hyperplasia, a feathery degeneration of hepatocytes and foci of necrosis. The kidney showed a cloudy swelling of the tubular epithelium, glomerular congestion, glomerulonephritis, protein casts in tubules and infiltration of focal inflammatory cells in the connective tissue. (*Zool. Mag. 90: 189-194, 1981*)

A developing country like India has to be alert towards the problem of environmental pollution. Cadmium, along with lead and mercury, has recently gained notoriety with regard to heavy metals' tissue destruction. During cadmium intoxication, most of this metal is accumulated in the liver and kidney irrespective of its route of administration (Shaikh and Lucis, 1972; Nordberg and Nishiyama, 1972; Suzuki, *et al.*, 1972). The pathologic changes have been correlated with the hepatic concentration of the metal (Colucci, *et al.*, 1975). Morphologic alterations in liver and kidney have been described after chronic cadmium administration in mammals and birds (Friberg, *et al.*, 1971; Castano, 1971; Berry, 1972; Nishizumi, 1972), but very little work has been performed on acute exposure (Hoffmann, *et al.*, 1976). The results have briefly been reported elsewhere (Lal, *et al.*, 1978) after 24 hr of cadmium exposure.

In view of increasing importance of cadmium as an environmental pollutant, the present study is an endeavor to record the morphologic alterations in liver and kidney of commom Indian ground squirrel (*Funambulus pennanti*, Wroughton 1905) following acute exposure of cadmium.

Materials and Methods

Thirty male laboratory-bred squirrels with body weight 100 ± 5 g were reared in steel-wired cages ($63\times48\times40$ cm) on wheat flour bread and water *ad libitum* (Table 1). These animals were divided into two groups: the first group comprising of 9 animals served as control, whereas, the second group of 21 animals served as test group and received single intraperitoneal injection of cadmium acetate (8.0 mg/Kg of body weight dissolved in 5% dextrose). The control group was treated with 5% dextrose intraperitoneally. Three animals from the control and 7 from the test group were sacrificed by decapitation following light ether anaesthesia after 1, 3 and 7 days. Post-mortem pieces of the liver and the kidney were

processed for routine paraffin sections (3-4 μm in thickness) and stained with hematoxylin-eosin. Proteins were localized by mercury-Bromphenol Blue method (Pearse, 1968). Liver and kidneys were weighed after termination of the experiment. Statistical analysis of the results was calculated by the method of Fisher (1950). The experiment was repeated twice to confirm the findings.

Results

Effect of cadmium acetate exposure revealed weight gain in liver and kidneys (Table 2).

Liver showed dilatation of central vein and Kupffer cells hyperplasia 1 day after injection. However, cloudy swelling of hepatocytes, feathery degeneration and areas of focal necrosis were pronounced from 3 to 7 days.

Table 1. Composition of wheat-flour bread. This diet was given in the from of *chapatis*.

Wheat flour	64 parts
Powdered gram	20 parts
Casein (dry powder)	12 parts
Sodium chloride	1 part
Calcium carbonate	2 parts
Vitamin mixture*	1 part

* Provides (in mg/100 g of diet): Biotin, 0.03; Folic acid, 0.4; Cholin chloride, 400.0; B_1, 2.4; B_2, 8.0; B_6, 2.6; C, 61; E, 100, K_3, 10.2; A, 9.6×10^{-4}; D_3, 2.85×10^{-6}

Table 2. Effect of cadmium on liver and kidney weight in squirrels.

Groups	Organ weight (g) after 7 day Liver	Kidney
Control	5.54 ±0.42	1.14 ±0.07
Cd Treated	6.82* ±0.67	1.96* ±0.14

Values are expressed in mean and represent a pooled data
* significantly different from the control ($P<0.05$) ± standard error

Focal collection of chronic inflammatory cells was observed in the vicinity of necrosed areas (Fig. 1).

One day after cadmium administration, marked congestion of renal parenchyma as well as glomerular components, besides cloudy swelling of the tubular epithelium was noticed. Mild glomerular congestion, marked degree of cloudy swelling of tubular epithelium, denudation and destruction of tubular epithelium were observed after 3 days. Intraluminal protein casts were seen in some areas. The nature of protein casts was confirmed histochemically. The blood vessels did not show any significant change. Connective tissue was found to be normal except for mild chronic inflammatory response. The picture simulating idiopathic membranous glomerulonephritis was observed 7 day after cadmium injection. Glomerular congestion accompanied by thickening of basement membrane was well marked. Cloudy swelling of the tubular epithelium, intratubular protein casts and focal inflammatory reaction in the interstitial tissue was also noticed (Fig. 2, 3) after 7 days of the metal exposure. No significant change in the blood vessels could be observed, except some scattered areas of peritubular and intratubular haemorrphage.

Discussion

Present observations showed increase in liver and kidney weight after a single cadmium acetate injection. Wilson *et al.*,, (1941) reported similar finding in cadmium exposed albino rats. Histology of the two organs revealed inflammatory reaction among hepatocytes and cloudy swelling of tubular epithelium and intraluminal protein casts in renal tissues respectively.

It is well established that regardless of the route of administration, hepatic concentration of cadmium via blood stream is very rapid and one of the highest in magnitude as compared to other organs (Lucis, *et al.*, 1960; Flick, *et al.*, 1971; Stowe, *et al.*, 1972; Moosl, *et al.*, 1973). Although the general plan of liver histology did not alter considerably during the

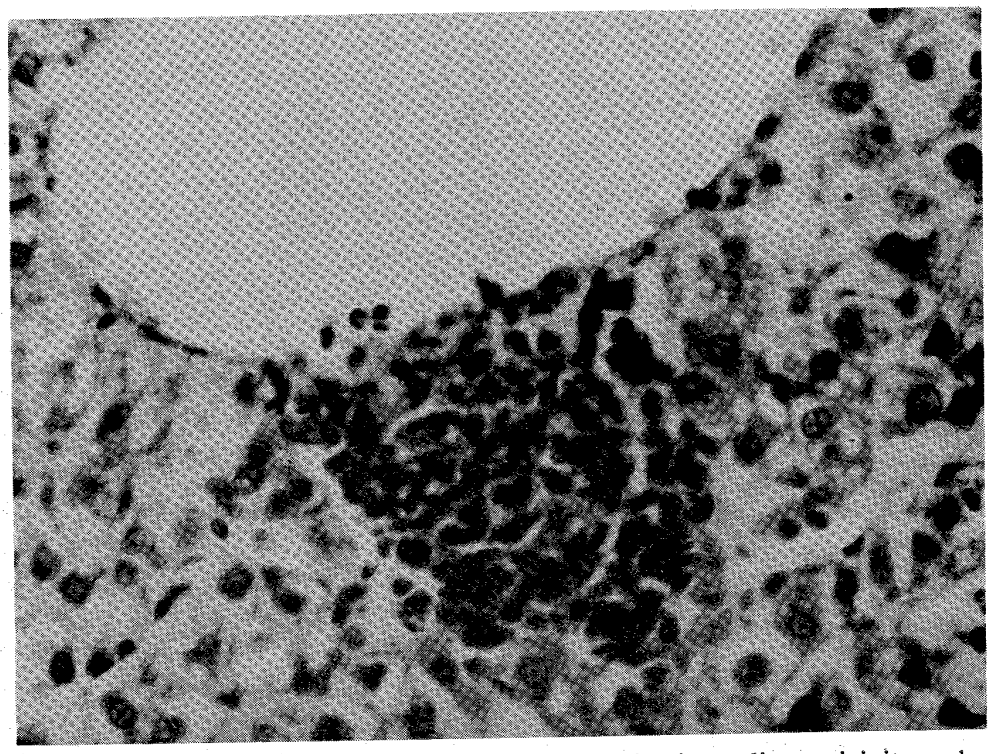

Fig. 1. Dilatation of central vein, focal necrosis in its immediate vicinity, cloudy swelling and feathery degeneration of hepatocytes are clearly observed. ×280.

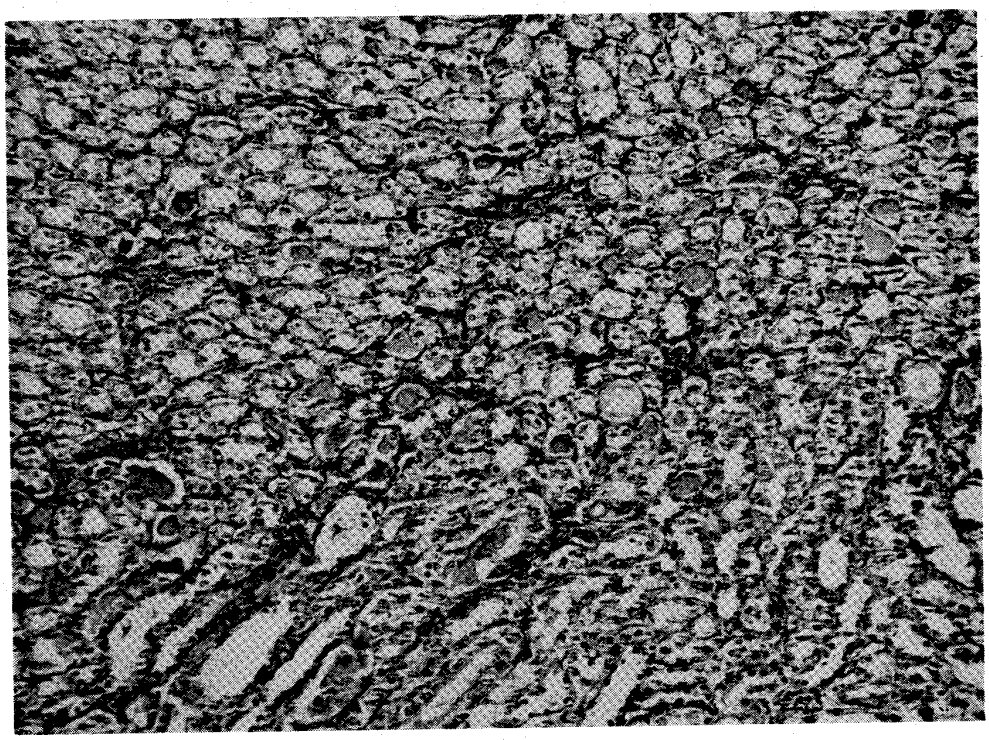

Fig. 2. Thyroid-like follicles reveal protein casts in the tubules. ×70.

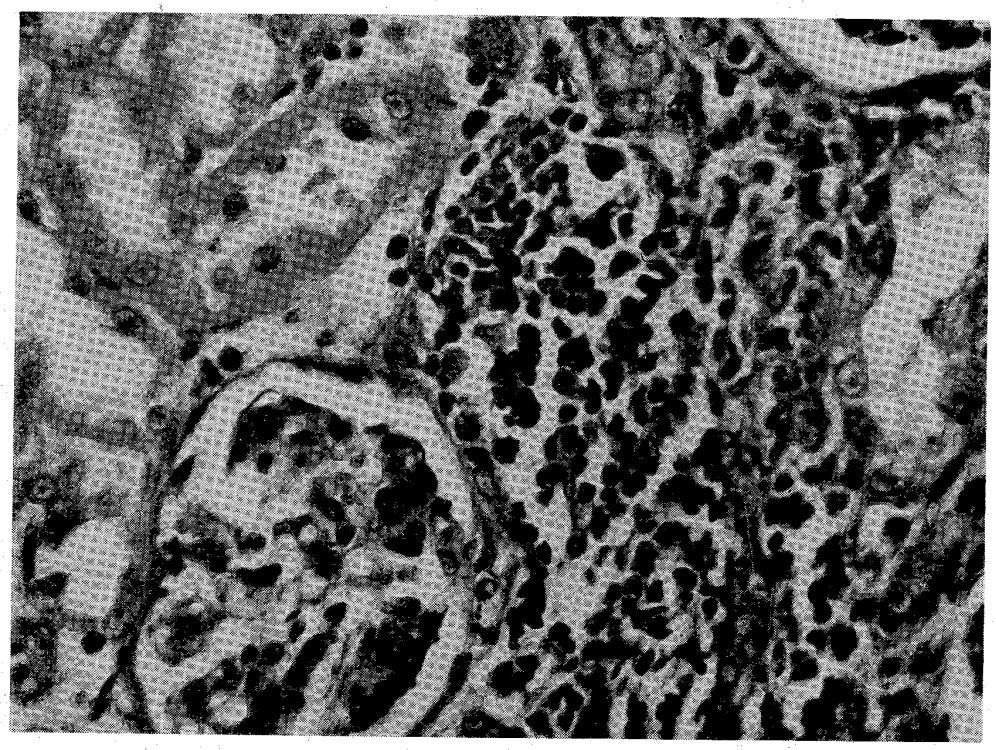

Fig. 3. Marked degree of glomerular congestion together with thickening of basement membrane, cloudy swelling of tubular epithelium and infiltration of focal inflammatory cells in the connective tissue are evident. ×280.

entire course of study, changes in the form of dilatation of central vein and Kupffer cells hyperplasia were recorded as early as first day after cadmium injection. The changes might be suggestive of underlying inflammatory and/or immunological response (Asvadi and Hayes, 1978; Metchnikoff, 1969) towards cadmium intoxication.

In kidney, besides perceptible congestion of entire structure including glomeruli, marked degree of cloudy swelling of the tubules was noticed from one day after the injection and onwards. The tubular epithelium tends to accumulate cadmium (Lucis, *et al.*, 1960; Berlin and Ullberg, 1963; Moosl, *et al.*, 1973; Hammer, *et al.*, 1973; Sugawara and Sugawara, 1974). Kawai and Kimura (1975) reported gradual development of generalized cloudy swelling of proximal convoluted tubules during first week after a single injection of cadmium chloride in rabbit. The present findings are in agreement with those of Itokawa, *et al.*, (1978), who reported degenerative alterations in kidneys which were characterized by cloudy swelling in the cells of tubular epithelium of proximal tubules in cadmium administered rats.

The hepatocytes revealed feathery degeneration after 3 and 7 days. The distribution of cadmium in different parts of liver has been described both after acute (Berlin and Ullberg, 1963; Friberg and Odeblad, 1957) and chronic exposures (Friberg, 1952). These studies suggested that cadmium is either equally distributed in the different parts of liver lobules or concentrated in the periphery of the lobules. Feathery degeneration of hepatocytes in the present study seems to be in agreement with the equal distribution of the metal because of the appearance of this lesion throughout the lobules. This lesion was also recorded in hepatocytes of Indian children (Mehrotra, *et al.*, 1975) and squirrel (Mithal, *et al.*, 1979). Progressive degeneration and necrosis of hepatocytes were recorded on 3rd and 7th day

of cadmium treatment. The cytoplasm became dense and opaque and lost its subtle granuarity. The foci of necrosis after 3 and 7 days suggest toxic action of cadmium on hepatocytes. Hoffmann, *et al.*, (1976) consider that irritation or toxicity produced by cadmium in liver cells might be responsible for morphological alterations.

The renal tubules revealed denudation and destruction of tubular epithelium after 3 days of cadmium treatment. Some of the tubules showed histochemically confirmed intraluminal protein casts. Cadmium has been found to cause proteinuria both in experimental animals and human beings (Piscator, 1966; Nomiyama, *et al.*, 1973). Proteins of low molecular weight, which can not be reabsorbed by the renal tubules are excreted in urine. The failure to reabsorb the low molecular weight protein is due to damage caused by the accumulation of cadmium in the renal tubules. It may be visualized that the appearance of intraluminal protein casts showed the possibility of proteinuria in these animals. The thickening of glomeurlar basement membrane observed after 7 days suggests the appearance of idiopathic membranous glomerulonephritis. This lesion of glomeruli seemed to be a sequel to the increased permeability, exudation and precipitation of plasma proteins between the basement membrane and epithelial cells. Itokawa, *et al.*, (1974) suggested that the glomerular lesion appeared as a secondary change following tubular dysfunction resulting from cadmium intoxication. Nomiyama and Nomiyama (1976) found that it was difficult to conceive the histological and functional changes in the glomerular membrane as being responsible for significant increase in the cadmium clearance. The connective tissue of the kidney after 7 days was infiltrated by focal collection of inflammatory cells, mainly lymphocytes. The inflammatory reaction was thought to be the outcome of cadmium toxicity. Focal inflammatory reaction was also observed in the connective tissue of renal cortex in rats (Sugawara and Sugawara, (1974).

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