30 min. The contents were treated with petroleum ether till nitrobenzene was completely eliminated and the residue was decomposed by warming with aqueous sulphuric acid (4%; 30 ml) on a steam-bath for 15 min. The demethylation product was extracted with ethyl acetate, then purified and isolated by preparative TLC using silica gel as an adsorbent and butanol:acetic acid :water (4 : 1 : 5) as the solvent system. I thus obtained crystallised from methanol as yellowish cubes, m.p. 295–96°, analysed for $C_{16}H_{10}O_7$ and gave positive Gibbs and Labat tests. UV (λ_{max}, MeOH): 260, 270, 350 nm; + $AlCl_3$: 280, 410 nm; + $AlCl_3$ + HCl : 270, 385, 410 nm.

Methylenation of 3,5,6,7,4'-*pentahydroxyflavone* (*II*)

3,5,6,7,4'-Pentahydroxyflavone (II) (1·0 g), methylene iodide (0·3 ml), potassium carbonate (3 g) and DMF–acetone (1 : 3; 100 ml) were refluxed for 50 hr and the progress of the reaction was monitored by TLC. The methylenation product I when worked up as described earlier, gave I which crystallised from methanol as yellowish cubes (0·15 g), m.p. 295-96°. I thus obtained was methylated with dimethyl sulphate and potassium carbonate in acetone to obtain its trimethyl ether (VI).

(I) and its trimethyl ether (VI) on direct comparison were found to be identical with the corresponding compounds obtained by the earlier method.

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SOME BIOLOGICAL, HAEMATOLOGICAL AND HISTOLOGICAL OBSERVATIONS IN MOLYBDENOTIC RATS

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ABSTRACT

Toxic effects of molybdenum have been experimentally established in rats. Physical examination of molybdenotic rats revealed a change in the colour of fur and retina. A rapid fall in the body weight and skeletal growth of molybdenotic rats showed its adverse effects on growth. However, the RBC, percentage of haemoglobin and haematocrit have been found elvated after molybdenum treatment. Histopathological studies on liver and kidney reflect the injurious effects.

INTRODUCTION

MOLYBDENUM (Mo) present in low concentrations in all tissues and fluids of all species plays an essential role in the animal and plant nutrition[1]. The industrial uses of molybdenum are well-known[2]. In molybdenotic rats, deficient lactation, male sterility and testicular degeneration have been observed[3]. In young rabbits molybdenotic syndrome is characterized by alopecia, dermatosis and severe anemia with a deformity in front legs[4]. Molybdenum is not acutely toxic in low concentrations[5]. A biological approach has now been made with histological and haematological parameters to study the conditions under which molybdenum participates in pathological processes in rats.

Materials and Methods

Blood cells, liver and kidney were selected for the present studies. A group of twenty male adult rats, *Rattus rattus* (albino), of the same age (90 days) and weight (90–110 gm) were fed on laboratory diet (Hindustan Lever Ltd.) and water *ad libitum*. After a few days, they were divided into two groups. Rats of the group A received (1·0 gm/kg body weight) ammonium molybdate daily in addition to regular diet for twenty days (ingestion of 400–800 mg Mo causes chronic toxicity)[6], while rats of group B were fed on lab. diet alone. The present dose was employed after determining oral LD_{50} for Mo (1·5 gm/kg body weight/day). Total weight of the diet was kept constant throughout the experiment.

During the experiment, regular recording of the body weight and skeletal growth was made. Before killing on the 22nd day, blood was collected by venipuncture from the rats of both the groups and processed for the determination of total RBC, percentage of haemoglobin[7] and haematocrit value[8]. After killing (exsanguination), the wet weight of the liver and kidney was recorded and the material was dehydrated at 100° C and the percentage of moisture was calculated. Small pieces of liver and kidney were processed for routine histopathology. In addition to haematoxylin/eosin staining, silver impregnation technique[9] and picric acid fuchsin[10] were also employed. Student '*t*' test was used to determine the statistical significance between test and control groups[11]. Results are presented as mean ± S.E.

Results

In Mo treated rats, no change in the colour of paws was noticed. However, fur was yellowish and the retina changed from pink to reddish brown in colour.

A rapid fall in the body weight and check on the skeletal growth were witnessed in molybdenotic rats accompanied by a rise in RBC. Percentage of haemoglobin and haematocrit values showed non-significant changes in Mo treated rats. Percentage of moisture in both the liver and the kindney was enhanced after Mo treatment (Table I).

Histopathological results reveal perilobular necrosis clumping of the nuclei in the central region and increase in the nuclear volume with conspicuous nucleoli in the liver (Fig. 1). Binucleated cells and simultaneously disappearing nuclei are the result of toxic abuses to liver parenchyma and changes in the physical and chemical characteristics of nuclear chromatin (Fig. 2). Portal haemorrhages were uncommon. However, the number of kupfer cells increased. Liver from the control rats exhibited normal histological picture (Fig. 3).

TABLE I

Mean values of body weight, skeletal growth, haemoglobin, haematocrit and RBC in the blood and moisture percentage in liver and kidney of molybdenum treated and control adult albino rats

Contents	Control	Molybdenum treated	Statistical difference
Initial body weight (g)	105±1·8	103±2·7	− 1·50
Final body weight (g)	140±3·6	60±2·0	+53·17
Initial skeletal growth (cm)	14·5±1·0	14·0±0·9	− 0·41
Final skeletal growth (cm)	16·5±1·9	14·3±1·2	− 1·10
Haemoglobin conc. g%	13·8±1·8	14·4±2·2	− 0·47
Haematocrit %	41·0±2·3	43·0±4·1	− 1·25
RBC No. 10^6/mm^3	8·8±0·3	10·2±0 3	+ 2·86
Liver water contents (%)	70·5±2·0	71·9±2·7	− 0·47
Kidney water contents (%)	74·4±1·2	80·2±1·0	+ 4·11

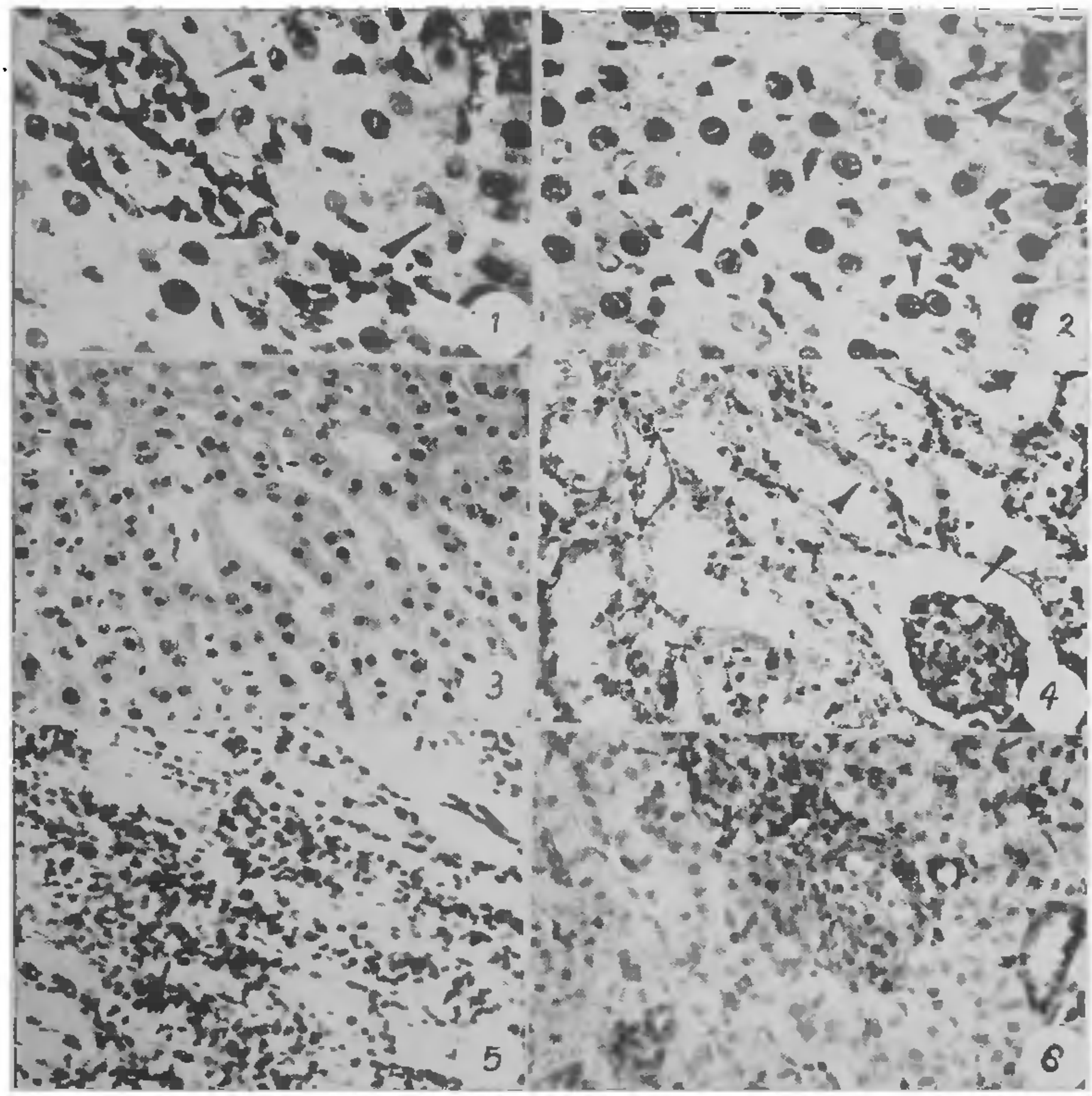
All values are the mean ± S.E. of 5 observations.

Present results show a higher degree of damage to the kidney as compared with the liver. Kidney of Mo treated rats showed glomerular shrinkage. Major injury was observed in the epithelium of proximal and distal renal tubules (Fig. 4). Medullary cells exhibited nuclear pycnosis (Fig 5). However, control kidney showed the intact renal morphology (Fig. 6).

In control liver, reticulin binds hepatic sinusoids to give them a firm structure (Fig. 7). Liver of molybdenum treated rats showed the accumulation of reticular fibres around the central vein (Fig. 8). In control rat kidney, thin and regular reticulin fibres encircled the renal tubules (Fig. 9). Kidney of Mo treated rats showed intertubular and irregular branching of comparatively thick reticulin fibres (Fig. 10).

Discussion

Growth retardation and loss of body weight after molybdenum intake have been reported in the past by several wokrers[6,12]. Present results show the Mo intake employed can also cause growth retardation. Impaired growth has been reported in lambs[13] fed on



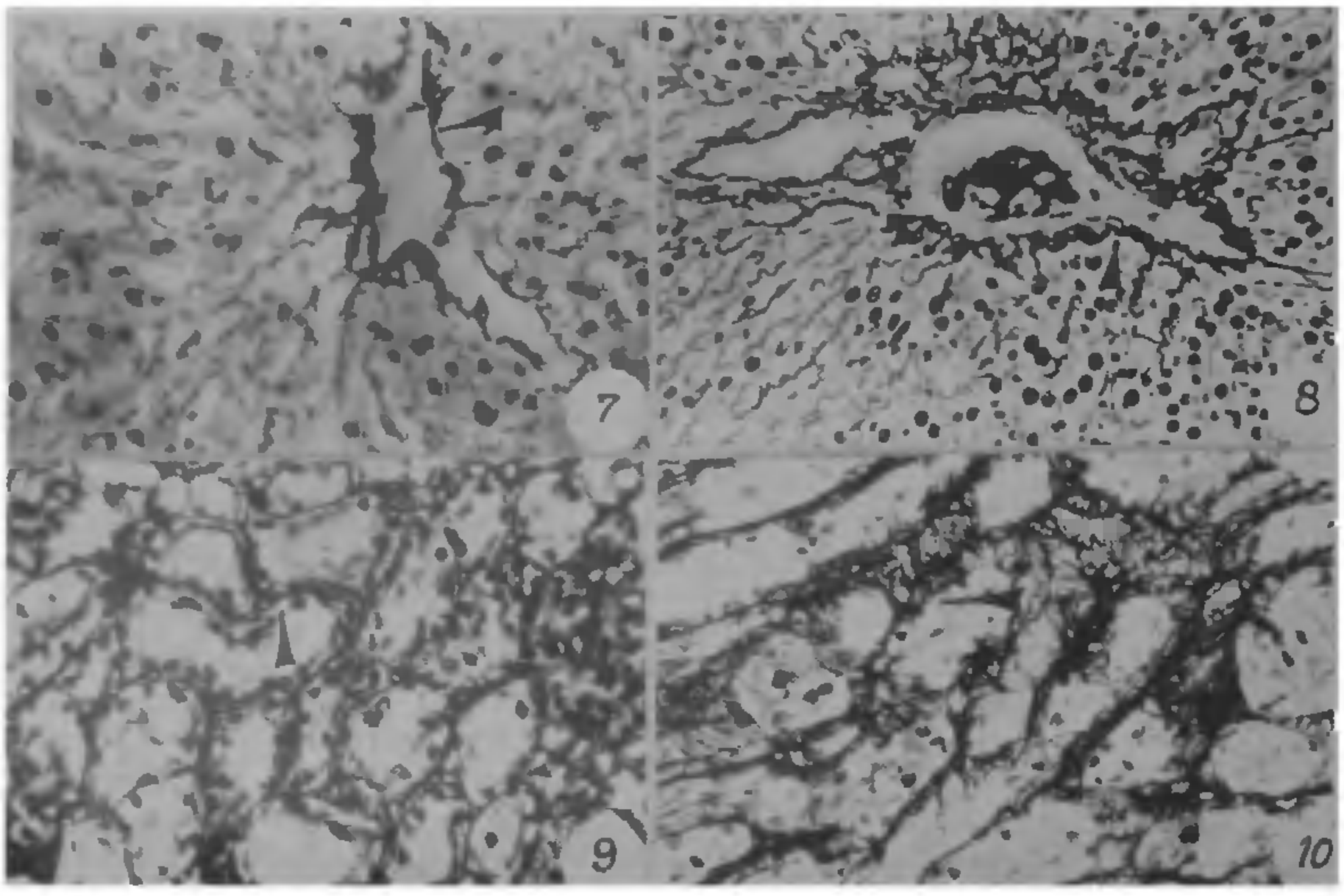
FIGS. 1–6. Fig. 1. T.S. of the liver from a molybdenum fed rat shows perilobular necrosis and nuclear pycnosis near the central vein, × 320. Fig. 2. Molybdenum treated rat liver also developed binucleated parenchymal cells with disintegrated nuclei, × 320. Fig. 3. Section of the liver from control rats exhibited intact parenchyma, × 320. Fig. 4. A section of kidney from molybdenum treated rats shows glomerular shrinkage and tubular epithelial damage, × 320. Fig. 5. Clumping of the nuclei in the medulla of kidney was caused by molybdenum, × 320. Fig. 6. Kidney of control rats showed renal tubules without any lesions, × 320.

forages containing 360 mg Mo/g. Depression in the growth rate is suggestive of the involvement of biological functions. Homeostatic mechanisms seem to be overcome by excessive exposure to Mo.

Increase in the number of RBCs shows a favourable effect contrary to harmful effect on the body weight and skeletal growth. Non-significant changes in haematocrit value and haemoglobin contradict the previous observations[4] who claimed that in young rabbits molybdenotic syndrome is characterized by severe anemia. At the present dose level, the molybdenosis does not cause anemia.

Adverse effect of molybdenum on liver and kidney became apparent when dry weight of these tissues were found remarkably decreased. However, moisture content increased in liver and kidney of Mo fed rats. One of the possible reasons for the increase in moisture contents would be impaired 'sodium-pump' mechanism leading to osmotic swelling[14].

Histology of liver and kidney points out the injurious effects of Mo on cell. Formation of a few binucleated cells are the indication of cell division occurring after Mo treatment. Necrosis seems to be the result of dysenzymia caused by Mo, since Mo competes with other trace metals essential for enzymes[15]. However, loss of nuclear chromatin as evidenced by the present histopathologial studies indicates loss of nucleotides. In molybdenotic rats degradations of nucleotides,



FIGS. 7–10. Fig. 7. Regular distribution of reticulin fibres is seen in the section of a liver from control rat, × 240. Fig. 8. Molybdenum treated rat liver exhibited thick reticulin fibres around the central vein, × 240. Fig. 9. In the kidney of control rat reticular fibres encircled the renal tubules, × 240. Fig. 10. Intertubular arrangement of thin reticulin fibres was exhibited by molybdenum treated rat kidney, × 240.

owing to impaired energy metabolism might lead to loss of purines from the cell. Thus, the sites of lost nuclei are the index of disturbance caused by Mo in liver.

Mechanism of renal damage by excessive Mo intake remains debatable. Involvement of the kidney in the increased excretion of uric acid derived from ele-mented purine degradation may be one of the reasons. Dysenzymia noted in kidney[16] may be another factor in damage to renal morphology.

Present results thus show that Mo, though essential for biological process as a trace element in the body causes damage to the system at elevated levels.

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