

# Morphofunctional Changes in the Kidneys Affected by Increased Concentration of Molybdenum in the Environment

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**Abstract** – The morphological and physiological state of the kidneys in experimental rats treated with molybdenum in the amounts of 0.0255 mg/kg, 0.425 mg/kg and 1.125 mg/kg was studied. Experiments were conducted on white laboratory rats of the Wistar line. The studies were conducted using histological, polarographic and mathematical methods. Sample molybdate sodium was prepared expressed metal. The experiment was carried out for 30 days. Histological examination of sections revealed significant pathological changes in the renal parenchyma. In the glomeruli of the cortical layer of experimental animals of the second series, anemia was observed: the capillaries are empty, they do not contain red blood cells. The tubular lumens are dilated, edemas; lymphostasis were observed in the stroma of the pyramids, and there was pronounced stagnation of blood in the direct veins. In epithelial cells, small-drop fat degeneration was observed. High concentrations of molybdenum causes hypertrophy of the kidneys, compaction of the vascular glomerulus, expansion of the cavity of capsules, narrowing of the proximal nephrons, poor circulation. A tenfold increase in dose causes the necrosis of the nuclei of endothelium and mesanglia, foci of necrosis, multiple point hemorrhages.

**Keywords** – kidney pathology, ecology, molybdenum, tissue hypoxia, molybdenum-containing enzymes

## I. INTRODUCTION

The body of a healthy person has a good self-regulating system of homeostasis, in which microelements (ME) play a certain role. Their level in the blood and tissue fluid is subject to physiological patterns. For most MEs, the main regulatory mechanisms of homeostasis are processes of absorption in the gastrointestinal tract, as well as their excretion with feces, urine, bile, sweat, milk, and deposited in the depot. According to some authors, molybdenum is absorbed into the blood in the small intestine, and the bulk is excreted through the kidneys [1–5].

In Kabardino-Balkaria, there is local environmental pollution with molybdenum-containing technological waste. Through the soil-water-plant-animals chain, an excess amount of molybdenum can enter the body of animals and humans. With excessive intake of ME, the elimination system comes into effect. The defect of any link in the system can disrupt homeostasis and cause diseases [4–9].

The purpose of the article is to study the morphophysiological state of the kidneys of experimental rats treated with molybdenum in the amount of 0.425 mg/kg,

which was found in places of local environmental pollution with man-made molybdenum production wastes.

## II. METHODS AND MATERIALS

To identify the role of Mo in developing morphological disorders, we conducted three series of experiments to study the effect of physiological doses of molybdenum, toxic doses, and effects at a 10-fold excess of the norm. Experiments were conducted on white laboratory rats of the Wistar line. For each of the three series of experiments, 2 groups consisting of 10 animals were formed. The first group was control. The rats were fed with routine food. The second group was experimental. In the first series of experiments, animals additionally received os per molybdenum at a concentration of 0.0125 mg per kg weight, in the second series – 0.425 mg per kg weight, and in the third series, animals received 1.125 mg molybdenum per kg weight. Sample molybdate sodium was prepared equivalent metal. The experiment was carried out for 30 days.

At the end of the experiment, the animals were decapitated. One of the kidneys was fixed in a 12% formalin

solution and subjected to the usual histological processing. Sections 5–7  $\mu\text{m}$  thick were stained with hematoxylin and eosin. The second kidney was fixed in a Becker fluid. Sections 10  $\mu\text{m}$  thick were prepared using a freezing microtome and stained with Sudan III according to Duddy.

As mentioned above, the main route for removing molybdenum from the body is through the kidneys. Therefore, after removal of the kidneys of animals, the content of molybdenum in the parenchyma was immediately determined using the polarographic method.

## III. RESULTS

The first series of experiments was aimed to identify the role of Mo in developing morphological disorders.

In animals of the control group, the structures of the renal corpuscles and tubules, connective tissue components and the degree of vascularization were normal. The connective tissue capsule was moderate. The stroma of the kidney was represented by loose, fibrous connective tissue, rich in reticular cells and fibers. The kidney parenchyma has a typical structure for this organ (Figure 1).

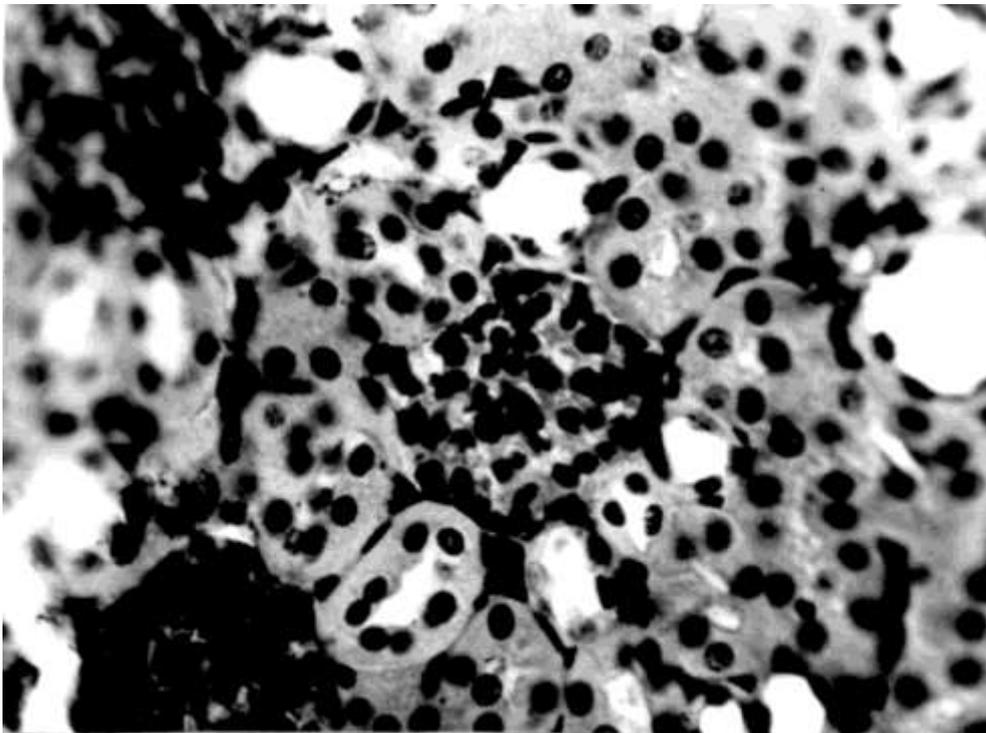


Fig. 1. Histostructure of the kidneys in the control group of animals (mag. of 7 x 40).

In this group of animals, the weight and concentration of Mo were  $11.6 \pm 0.052$  and  $0.0027 \pm 0.0001$ , respectively.

In animals of the second group receiving a physiological dose of Mo molybdenum, there were destructive changes in the stroma of the organ. There are expanded capillaries of the vascular glomeruli and the cavity of the capsule of the renal corpuscles. The gaps of the tubules in the cortical and

collecting tubes of the medulla are normal. Disruption of blood supply and fat metabolism were not observed. The weight of the kidneys and concentration of Mo were high.

When staining sections according to Duddy, these cells do not accumulate fat. The concentration of molybdenum of 0.0125 mg/kg weight does not cause significant structural changes. The moderate expansion of the glomerular capillaries and cavity of the capsule are signs of increased glomerular

filtration, which is a response to an increase in metabolic processes, including purine, under the influence of a physiological dose of molybdenum [5, 8]. Activation of metabolic processes occurs through molybdenum-containing enzymes (xanthine oxidase, aldehyde oxidase and sulfite oxidase), and by the indirect effect of Mo on the succinate dehydrogenase, catalase and alkaline phosphatase, hyaluronidase, glutaminase [1–3].

In the second series of experiments, to identify the effect of molybdenum toxic dose on the morphophysiological indicators of the kidneys of experimental animals, the following indicators were determined: Mo concentration in the control group –  $0.0022 \pm 0.0005$  mg %, organ weight –  $165.4 \pm 2.4$  mg, and  $189.3 \pm 1.3$  mg in mice from the territory of NHMP. Weight gain indicates organ hypertrophy and its plethora (Figure 2).

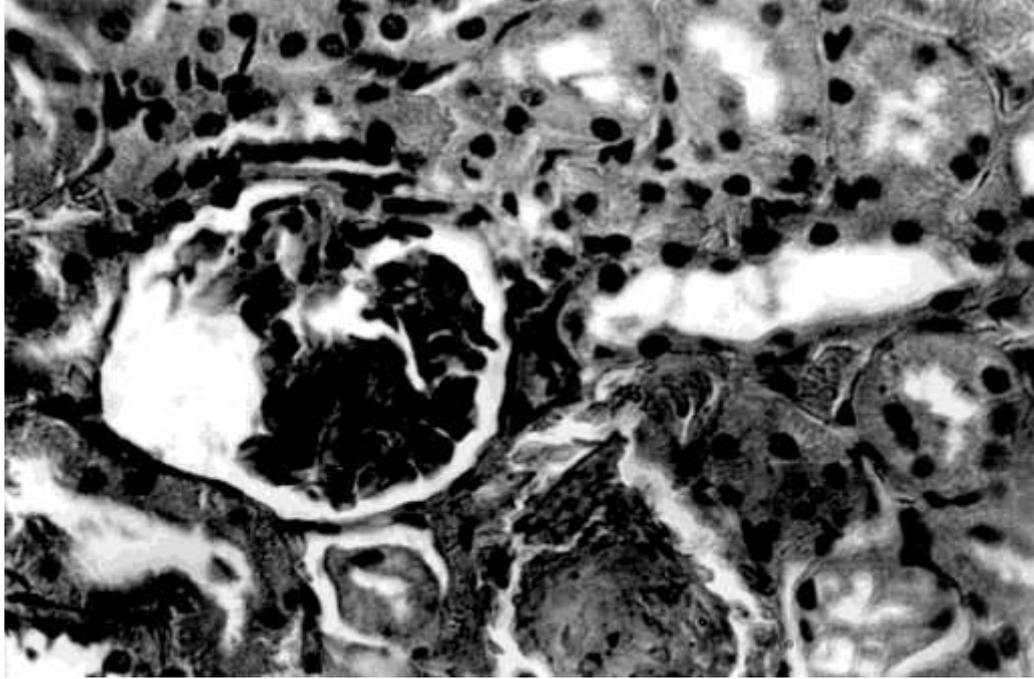


Fig. 2. Histostructure of the kidneys in forest mice from the territory of NHMP (mag. 7x40).

The analysis of rat micropreparations from the second experimental group identified such pathological changes as consolidation of the renal body, expansion of capillaries, proliferation of mesanglion, hemorrhages in the parenchyma and lymphoid infiltration.

In almost all preparations, there is an uneven blood supply to the organs, expansion of the lumen of the capsule and narrowing of the lumen of the proximal nephron tubule.

In the cortical substance, ischemic phenomena are more pronounced, and in the cerebral substance, blood circulation is increased. This speaks for redistribution of blood in the kidney due to the narrowing of the vessels of the cortical layer and the discharge of the main blood at the border of the brain and cortical layers.

The observed expansion of the capsule is possible due to narrowing of the proximal tubules (there are tubules with a confluent lumen) and, as a result, the hard outflow of primary urine.

The vascular glomerulus was hyperchromatic, the cells were swollen, the nuclei were hyperchromic. In individual pyramids, foci of karyorexis and karyolysis were observed (Fig. 3).

In the parenchyma between the tubules, there were small hemorrhages. When processing the sections with black Sudan, small-drop fatty degeneration of the tubule epithelium was observed.

Thus, in rats of the experimental second group, hypertrophy of the kidneys, an increase in the content of molybdenum, compaction of the vascular glomerulus, expansion of the capsule cavity, narrowing of the proximal nephrons, hemorrhage, and circulatory disorders were observed.

These phenomena are due to the following mechanisms:

1. An increase in the concentration of molybdenum above the threshold inhibits the work of the thyroid gland, adenohypophysis, whose hormones are involved in fat metabolism. The lack of thyroxin causes a delay in the utilization of fat and its deposition in the organs.

2. Fatty dystrophy contributes to molybdenosis - anemia which causes tissue hypoxia. Based on the literature data, during anemia, destructive changes impair fatty acid oxidation in the cells which causes fatty degeneration. Localization of dystrophic phenomena in the proximal and distal nephrons is

due to the fact that in the conditions of hypoxia, organs and cells in functional stress are the first to suffer.

3. The influence of molybdenum on the food center is possible. Along with other parts of brain, neural formations (nuclei) located in the posterior hypothalamus and ventrolateral and ventromedial nuclei play an important role. Hypofunction of ventrolateral nuclei causes loss of appetite,

emaciation and fat utilization. Hypofunction of ventromedial nuclei causes obesity. Studies of the effect of molybdenum on the hypothalamus were carried out in our laboratory. According to them, inhibition of the anterior nucleus of the hypothalamus with increasing concentration of molybdenum in the body is possible. Inhibition of ventromedial nuclei contributes to fatty degeneration.

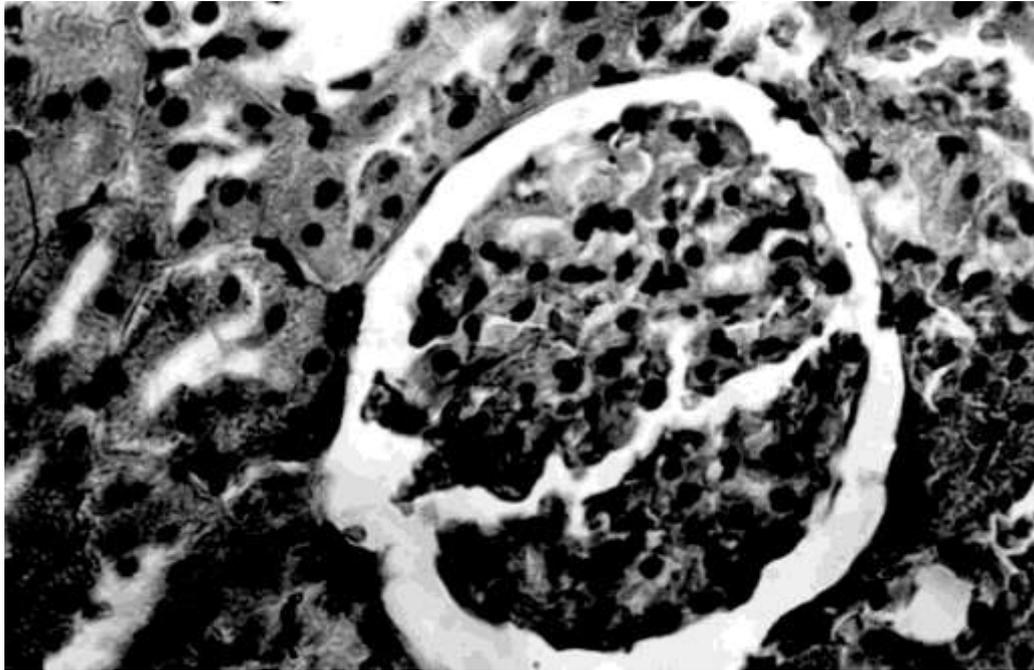


Fig. 3. Hypertrophy of the glomeruli of the kidneys in experimental rats (mag. 7x40).

On sections apart from the capillaries of the glomeruli, there are extensions of the larger vessels and veins.

In the parenchyma between the tubules, there are hemorrhages in the form of small foci and extensive formations. This is due to a violation of elasticity, and integrity of the fibers of blood vessels. Molybdenum violates elasticity of the fibers of blood vessels. There are growths of connective tissue layers and accumulations of lymphocytes.

The next series of experiments is devoted to studying the effect of increased (10 times) Mo content in the diet on the morphophysiology of the kidneys.

When studying the sections under the microscope, focal damage to the structures of an organ was observed. Hypertrophy of renal bodies was observed in lesions. Their diameter is  $55.2 \pm 1.3$   $\mu\text{m}$  (in the control animals, it is  $42.2 \pm 3.4$   $\mu\text{m}$ ).

However, there are many capsules where the vascular glomerus is tightly sealed. Some of them resemble "paws", while others are wrinkled. They are at different stages of degeneration. This is evidenced by necrosis of the nuclei of the endothelium and mesanglia. There are foci of necrosis, point hemorrhages.

When reviewing microscopic preparations, deeper disorders of vascularization in the medulla are noted. Polychromasia of the tubular epithelium was observed. The basis of

polychromasia is excessive intake of molybdenum. It is known that molybdenosis causes anemia, leading to tissue hypoxia due to impaired blood circulation (congestion due to varicose veins) and a decrease in hemoglobin in red blood cells [5, 7]. Our laboratory found a significant decrease in hemoglobin in these animals to 9.0–0.08 g% (in the control group – 12.0–0.4 g%).

Under these conditions, glycolysis is enhanced, and a lot of lactate is formed which results in non-respiratory acidosis, when pH inside the cell medium is below the optimum, which causes disruption of cellular metabolism.

When treating the sections with black sudan, focal small-fatty degeneration was observed. It is more pronounced in the epithelial cells of the medulla. This phenomenon is primarily associated with tissue hypoxia. With anemia, destructive changes occur in the mitochondria, leading to disruption of fatty acid oxidation which causes fatty dystrophy.

Another option is possible. Excess molybdenum causes imbalances in the neuroendocrine system which can lead to the same results. The weight of the kidneys and concentration of molybdenum are  $2.4 \pm 0.05$  g and  $0.0048 \pm 0.0005$  mg%, respectively.

Microscopic examination of sections of the kidneys of treated with 1.125 mg/kg of molybdenum revealed significant destructive changes in all the tissue elements of the kidneys.

There were necrotic changes in the parenchyma and glomeruli. Blood cells were observed in the lumen of the capsule which indicates a violation of the integrity of the capillaries. The nuclei of the endothelium and mesanglia are pyknotic; karyolysis is observed on the edge of the glomerulus. The tubular lumens are dilated, indicating stagnation. Small granularity is visible in the cytoplasm - a sign of fatty dystrophy. The glomeruli are visible, both with an expanded capillary network and a wrinkled vascular glomerulus. There are foci of necrosis and paranecrosis, hemorrhages, and lymphoid infiltration.

The structural changes in the kidneys are associated with a significant excess of Mo in the diet. The proposed mechanisms were used to explain the results of the previous series. The state of the organism in the previous series can be described as "resistance" according to Selye, when the organism is mobilized for survival. In this series, it can be described as "exhaustion" because the stimulus is too intense.

The basis of the structural changes in the kidneys in forest mice from the territory of NHMP is the damaging effect of excess molybdenum in drinking water and food. These damages are less pronounced due to different experimental conditions: in the diet of forest mice, molybdenum antagonists can be decreased reducing its toxicity; in laboratory experiments, animals received pure Mo; therefore, its concentration and the damaging effect was higher [2, 11].

#### IV. CONCLUSION

An increased intake of molybdenum in animals and humans can cause pathological changes in the structure and decrease the filtration capacity of the kidneys.

Our experiments and further analysis of the histological structure of the renal tissue identified morphological changes typical of acute renal failures. Acute renal failures can be caused by nephrotoxic substances (toxins, pharmacological agents, infectious agents of the urinary tract) and salts of heavy metals. Circulatory disorders in the cortical and cerebral layers of the renal tissue are due to excessive intake of salts of molybdenum. As a consequence, ischemia and hypoxia in the

kidneys were observed. The overall picture of acute renal failures is complemented by degeneration and necrosis of the glomeruli and kidney tubules resulting from impaired metabolic and redox processes.

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