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Cytomorphological Changes of Gepatorenalny System of Rabbits at The Combined Poisoning with Xenobiotics.

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ABSTRACT

Study of the combined effects of toxicants into the organism of mammals in the ultra-thin level represents an important scientific interest. The aim of our work was to study the combined effects of dioxin and lead acetate on cytomorphological liver structure and rabbit kidneys. To determine the characteristics of the joint effect of the toxins have a different nature and origin on the histogram cytostructure liver and kidneys, the methods of histology and electron microscopy, we have been put to combined experience in dioxin poisoning of rabbits and lead acetate in doses of 1/200 and 1/10 of the LD50. Analysis cytomorphological changes in the liver and kidneys showed the following pathological disorders: increased clearance between the hepatic beams, some sinusoids detected cellular detritus, chaotic arrangement of hepatocytes, more rounded than in the control, in some hepatocytes nucleus of irregular shape, with a shift to the periphery, vacuolization of cytoplasm, with signs of granular dystrophy, swollen capillaries of renal corpuscles, reducing the lumen of the convoluted tubules, granular dystrophy. Ultrastructural condition mitochondria (increase in size, illumination, and matrix swelling) confirms granular dystrophy, revealed by histological studies. In the cells of rabbit liver in combined toxicity of dioxin and lead acetate manifested pathological changes in the ultrastructure: violation of perinuclear space (TNG), vacuolization of cytoplasm, the destruction and fragmentation of the EPR, the destruction of the mitochondria. The results show marked histological effect of toxicants tested when combined admission to the hepatorenal rabbits system.

Keywords: Rabbits, hepatocytes, epithelial cells, dioxin, lead acetate.

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INTRODUCTION

It is generally known that as dioxins, heavy metals and have a wide range of negative effects on animals and humans [1, 3, 2, 12, 7, 17, 9]. However, in their natural habitat biological objects toxicological test load of a number of hazardous compounds [5, 11, 14, 16, 13, 10]. As pointed out by [15, 8, 4] risk assessment of chemical mixtures, the combined and cumulative impact in the development stage, ie this scientific base needs to be significantly expanded. Of particular disadvantage in knowledge about how to control the effects of complex exposures. Accordingly, the inspection and testing of the "model" of the combined effects on the animals, including histological and electron microscopic levels, has an undeniable interest to basic science.

METHODS, TECHNIQUES

The studies were conducted on the "Chinchilla" rabbits breed, reaching 9 months of age, body weight from 2 to 3 kg. For morphological studies, control and test specimens were taken from the experiment in accordance with "international guidelines for biomedical research using laboratory animals." We analyze the state of histological and ultrastructure of liver parenchyma cells and kidney cortex.

Pigs for experimental chronic intoxication was used:

- 2,3,7,8-tetrachlorodibenzo-para-dioxin (the dioxin), made "Khimprom" (Ufa) Bashkortostan;
- Lead acetate (C4H6O4Pb × 3H2O) - GOST 4426 - 75;

During the experiments maintained the same conditions and feeding of test and control animals, according to the zoo technical standards.

The experiment lasted 40 days. The first group was the control of biological and received a normal diet. Animals of the second group were combined daily oral inoculation 2,3,7,8-THDD in a dose of 0.15 mg / kg body weight, which corresponds to 1/200 of the LD50 of lead acetate and a dose of 1/10 LD50 (65 mg / kg) .

For histological tissue samples were fixed with 10% neutral formalin, then embedded in paraffin dehydration. Histological sections prepared using a microtome arch, Ehrlich stained with hematoxylin - eosin water. Photomicrography were performed at the facility: Microscope Leica DM 1000 digital camera Leica DFC 320 (Germany).

For electron microscopy, preparation of selected material held by the classical scheme [6]. Specimens were fixed in 1% glutaraldehyde solution (SERVA, Germany) with 0.1 M phosphate buffer (pH 7.4) 12 hours in a refrigerator. Postfiksatsiyu carried out in a 2% solution of osmium tetroxide (Moscow chemical plant) in the same buffer for 2 hours. After dehydration in alcohols material pieces of the samples were embedded in a mixture of eponovyh (Epon 812, DDSA, MNA, DMP-30) resin (Fluka). Ultrathin sections were prepared on the LKB III 8800 ultramicrotome, mounted on electron microscopic grids and stained with uranyl acetate (1 hour) and lead citrate (1.5 min). We studied in the electron microscope JEM 100 CX-II («Jeol», Japan). The shooting took place on phototechnical film AGFA ORTHOCHROMATIC. Digitization of negatives made by the scanner EPSON PERFECTION 4990 PHOTO 600 dpi. The end result - processed using ACD SeeProv.6 programs. and Axio Vision Rel. 4.8 (Carl Zeiss).

RESULTS AND DISCUSSION

When the light-optical study of histological sections revealed rabbit liver sections having a typical lobular structure (Fig. 1 a). In the liver slices positioned hepatic hemocapillars. In many sinusoids stored red blood cells, Kupffer cells are registered with dense cytoplasmic inclusions.

Hepatocytes are polyhedral cells that are located close to each other and each cell borders sinusoidal vessel. The central core of cells located in different sizes rounded. There are dual-core cells. In the cytoplasm logged dark-colored fat inclusion.

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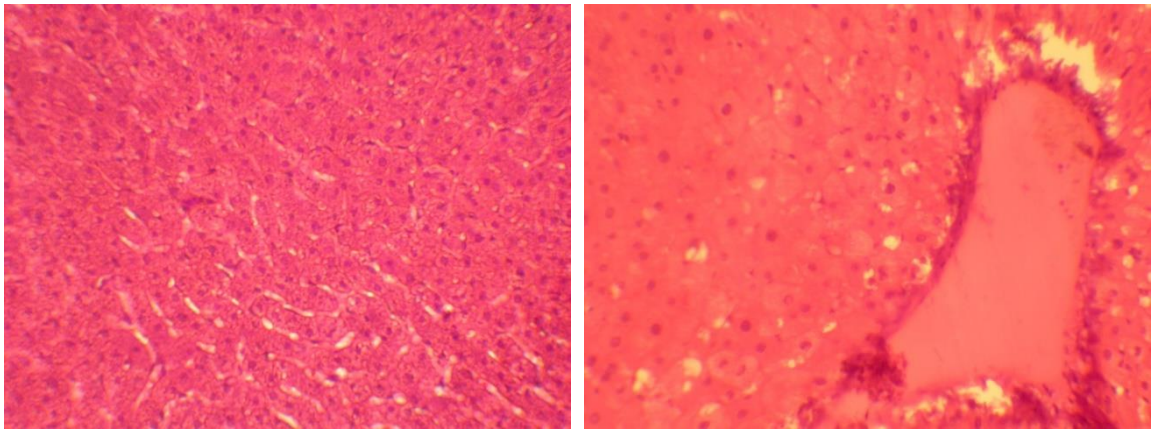

Fig. 1, b. Land of the liver: a) control group rabbit, b) the rabbit in receipt combines dioxin and lead acetate at doses of 1/10 and 1/200 of LD50 LD50 (lens 20).

Studying under a light microscope rabbit liver after exposure to a dose of lead acetate 1/10 LD50 at 40 days showed hepatic lobules consisting of trabeculae. There has been an increase in hepatic clearance between the beams, the central vein blurry boundary (Fig. 1 b). In some sinusoids detected cellular detritus, white blood cells with dense nuclei. Red blood cells are irregularly shaped and rarely recorded. Macrophages are more numerous than in the control animals. At the border there are some sinusoidal small-globular precipitation. Hepatocytes were randomly placed in the hepatic lobules, lost the right radial topography hepatic beams. Cells acquire a more circular shape than in the control. In some hepatocyte nuclei observed irregular shape offset toward the periphery of the cell. Many densely stained nuclei are likely to be on the stage pyknosis. The cytoplasm becomes foamy, vacuolated structure can be diagnosed granular dystrophy (Fig. 1b).

Thus, when rabbit liver histological study after exposure of lead acetate in 1/10 LD50 dose on day 40 revealed significant changes in liver tissues and cells: edema sinusoids, in the presence of cellular debris and hemocapillars central vein, small-globular precipitate circumferentially sinusoids granular dystrophy of hepatocytes and karyopyknosis in some cells.

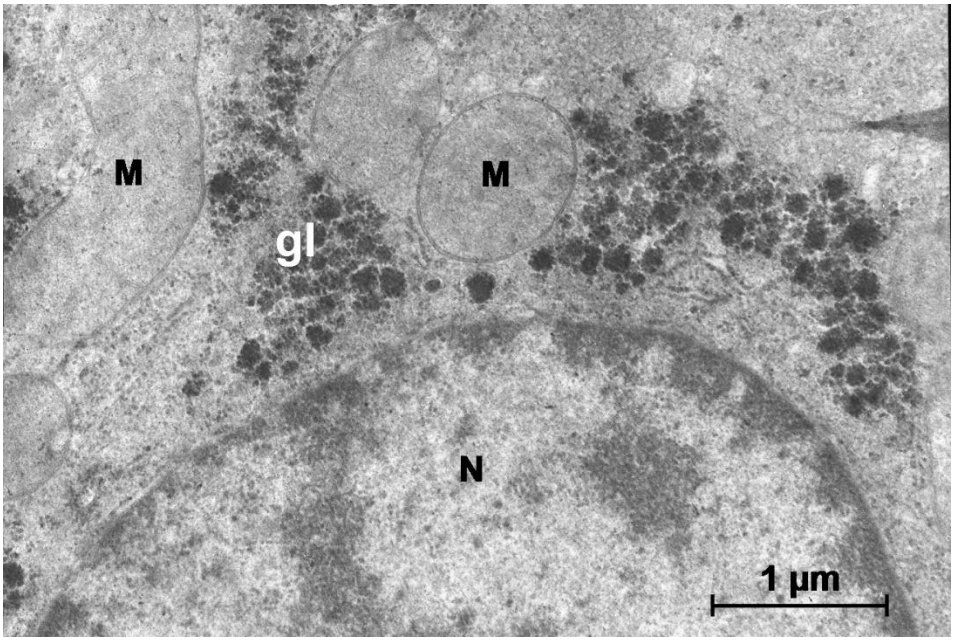

Fig. 2. A fragment of hepatocyte control group rabbit
Legend: N - the core of M - the mitochondria, gl - glycogen.

The ultrastructural study of the control group of rabbits liver samples revealed a large nucleus, located in the central part of the cell, it has a rounded shape with diffuse chromatin arrangement. Perinuclear space of uniform width around the perimeter of the nucleus, nucleoli are clearly seen, the pores in the nuclear envelope. Enroll dual-core cells. Mitochondria generally elongated shape with a lamellar cristae and dense matrix. Christa evenly fill the volume of the compartment, and are clearly seen. Intermembrane spacing evenly throughout their length. The cytoplasm of the average electron density. In the cytoplasm distributed large amounts of glycogen (Fig. 2), peroxisomes, Golgi apparatus. Smooth endoplasmic reticulum is well visualized throughout the cell volume, granular endoplasmic reticulum is developed in the perinuclear zone, with clearly visible ribosomes.

Hepatocytes rabbits after the combined effects of dioxin and lead acetate in doses of 1/200 and 1/10 LD50 LD50 have a nucleus highly modified form. Changing the packing of chromatin, a lot of densely packed chromatin at the periphery. The nucleoli are rare, there is little the nuclear pore. Perinuclear space (Fig. 3) in some places reaches a width of 390 nm. Matrix mitochondrial electron-dense, flaky. The membranes of mitochondria are rendered as blurry, Kristen observed mainly in the periphery. dense granules are visible in some of the mitochondrial matrix. It distributed throughout the cytoplasm very large number of small and large vacuoles with electron-transparent content of fat and glycogen inclusions little.

There have telolizosomy residual body and MLT. There granular endoplasmic reticulum with swollen tanks. Occasionally tracked channels smooth endoplasmic reticulum. Golgi hardly present.

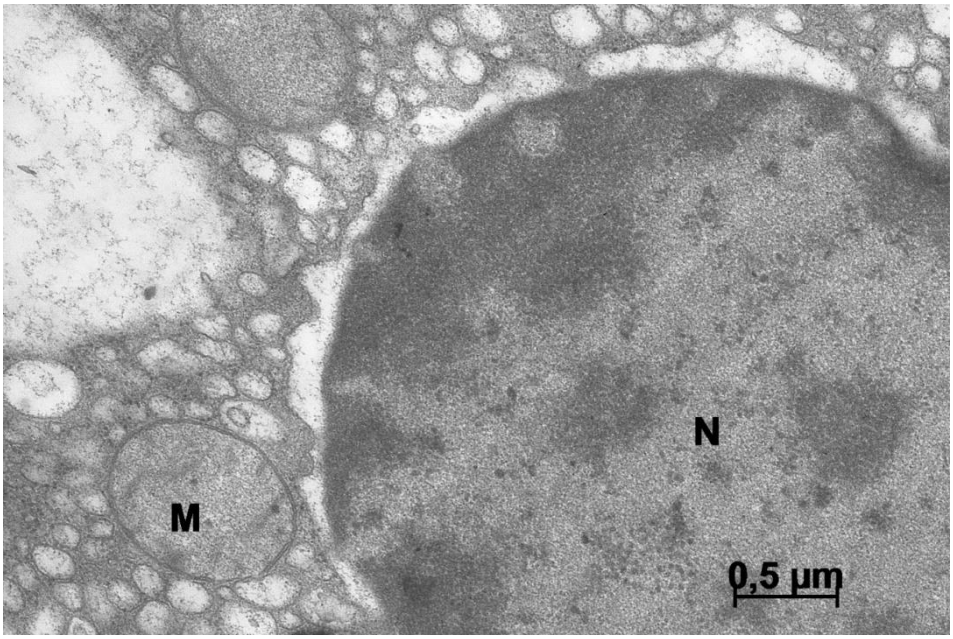

Fig. 3. Plot rabbit hepatocyte after combined exposure dioxin 1/200 LD50 dose of lead acetate and a dose of 1/10 LD50
Legend: N - the core of M - the mitochondria.

In the study of histological sections of the cortex under the light microscope observed renal corpuscles, which are woven within the capillaries surrounded by podocytes. Podocyte nuclei of large, visible nucleoli and peripheral chromatin. Around glomerular nephrons observed convoluted tubules (Fig. 4a). After the combined effects of dioxin in a dose 1/200 LD50, and lead acetate at a dose of 1/10 LD50 in the renal corpuscles diagnosed capillaries swelling and accumulation of blood cells. The cells surrounding capillaries - podocytes, have very dense core. Marked decrease in the lumen of the convoluted tubules (Fig. 4b). In nephrocytes recorded granular dystrophy, which is characterized by impaired energy balance. The nuclei of some epithelial cells of the proximal and distal tubules become dense structure and become smaller. In the cortex visualized areas of desquamation of epithelial cells of the convoluted tubules. The capillaries that surround the convoluted tubule, there is accumulation of red blood cells.

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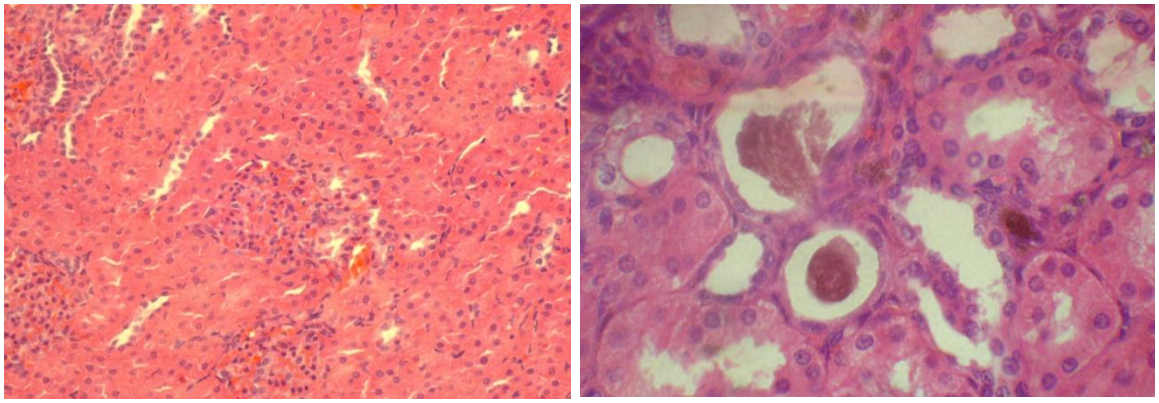

Fig. 4, b. Land kidney cortex: a) a control animal (lens 20); b) the rabbit after the combined effects of dioxin in a dose 1/200 LD50 lead acetate, and in a dose of 1/10 LD50 (lens 40)

Cortical substance of the kidneys of the control group of rabbits. Epithelial cells of the proximal tubules of the kidney. The core of the spherical form chromatin average electron density. Condensed chromatin is distributed over the periphery of the nucleus. Clearly visible nucleoli. Perinuclear space is the same width along the entire length of the nuclear envelope. The cytoplasm of the average electron density. Mitochondria are mostly oblong medium size (Fig. 5). The elongated mitochondria, which are located in close proximity to the folds of the cell membrane of the basal cells, reach a length of 4 m. Matrix moderate electron density. Christa clearly visible. Intermembrane distance cristae evenly over their entire length. Lamellar cristae companies in parallel rows. The folds of the plasma membrane of the basal cells have a high contrast and clearly visible. They protrude far deeper into the cells reach the epithelial cells of the nucleus. Rarely, the Golgi apparatus with small tanks and the endoplasmic reticulum. The cytoplasm is filled with a small amount of small vacuoles with the contents of various electronic density, which can be diagnosed as pinocytic bubbles. The apical part of the epithelial cells has a large number of microvilli with well-developed actin filaments. Microvilli greatly increases the surface of the cell membrane, thereby increasing the re-absorption process, which occurs in the proximal tubule epithelial cells.

In ultrathin sections of kidney cortex of rabbits in the control group recorded portions of the glomerulus, which processes podocytes surround capillaries and form the filtration barrier. Each podocyte has three parts: the "body" of the cell, primary and secondary processes. The main part of podocytes, we see the core with an average density of chromatin. Condensed chromatin is evenly throughout the volume of the nucleus. The core is surrounded by the nuclear envelope with nuclear pore. Close behind tsitopodii form on the surface of the basement membrane of the capillary slit diaphragm through which the ultrafiltration process. Basal filtration barrier plate has a three-layer structure consisting of a central dense plate, surrounded on both sides of the light weakly colored plates. The thickness of the basement membrane filtration barrier glomeruli of rabbits has an average of 278 nm. On the part of the capillary to the basal lamina adjacent to the endothelium Fenestra.

The cytoplasm of podocytes is filled with mitochondria, which in large quantities are located in the perinuclear region. They have an average electron density matrix. Christa mitochondria uniform. Often there are cisterns rough endoplasmic reticulum, free ribosomes and Golgi complex. The trabeculae clearly visible actin cytoskeleton.

Cortical substance of the kidneys of rabbits after the combined effects of dioxin and lead acetate in doses of 1/200 and 1/10 LD50 LD50. Epithelial cells of the proximal tubules of renal cortical epithelial cells have a nucleus round shape (Fig. 6). Chromatin average electron density. Chromatin often distributed evenly throughout the volume of the nucleus. The nucleoli are clearly distinguishable. Perinuclear space evenly along the entire length karyotheca. Cytoplasmic enlightened. Granular endoplasmic reticulum bad stink. The mitochondria of epithelial cells are either electron-transparent flake matrix with short cristae on the periphery or degraded Christi in the form of residual membranes. At the same time recorded small mitochondria with dense matrix and clear Christi. Mitochondria epithelial size from 0.5 to 2.5 microns. In the cytoplasm there are a large number of peroxisomes with electron-dense contents. Golgi apparatus does not meet almost. The folds

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of the basal plasmalemma rare, but extended. The smooth endoplasmic reticulum consists of small channels. In the cytoplasm are found throughout much of peroxisomes and telolizosom.

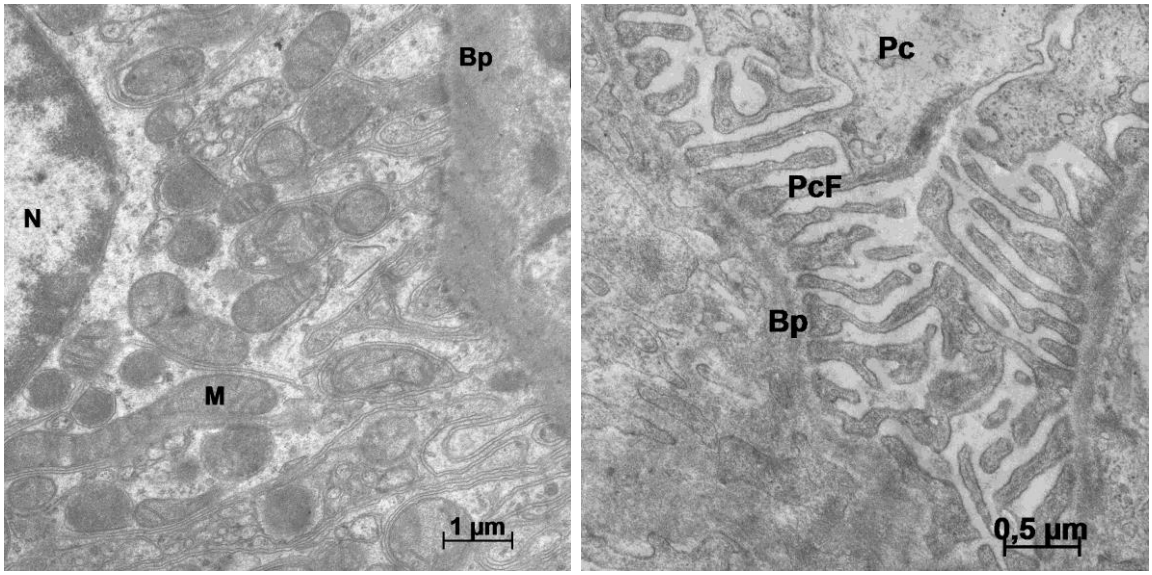

Fig. 5. Lots of cells of the cortex of the control group rabbit kidney: a) apical part of the epithelial cells of the proximal tubule; b) podocyte station and filtration barrier glomeruli
Legend: N - the core of M - the mitochondria, Bp - basal plate, Pc - podocytes, PcF - podocyte foot.

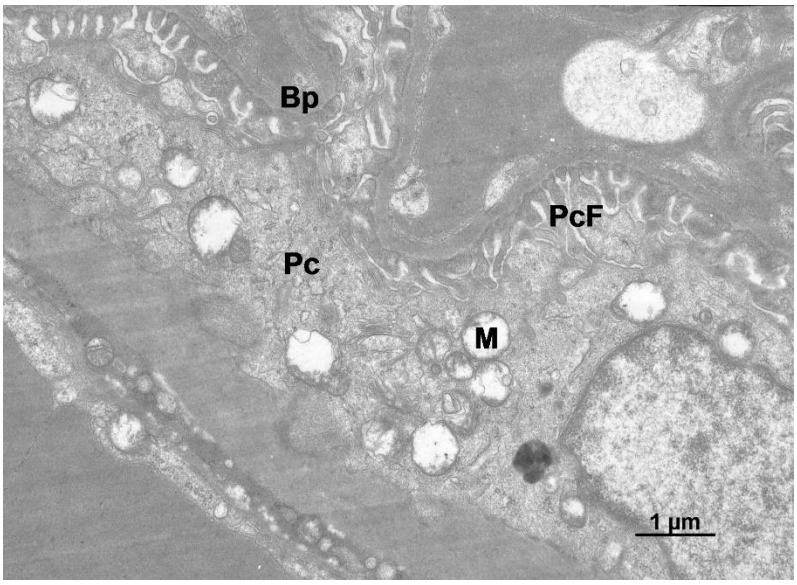

Fig. 6. Plot rabbit renal glomerulus after combined exposure dioxin 1/200 LD50 dose of lead acetate and a dose of 1/10 LD50.
Legend: N - the core of M - the mitochondria, Bp - basal plate, Pc - podocytes, PcF - podocyte foot.

If poisoning rabbits combines dioxin in a dose 1/200 LD50, and lead acetate at a dose of 1/10 LD50 observed change in glomerular filtration barrier, podocytes lose slit diaphragm and extend along the capillaries. Registers the change of the basement membrane. It is reduced in size and has an average thickness of 187 nm. Thick part of the membrane is loosened, it splits into separate fibers and loses its functional role. Many pedikuly podocytes lose their body increases in volume due to vacuolation, broken trabecular (Fig. 5).

Nuclei of cells of the renal glomerulus of irregular shape with a dense distribution of chromatin throughout and slight predominance karyotheca circumferentially. Mitochondria in podocytes is much less than in epithelial cells, they are electron-dense matrix and many revealed signs of degradation. The cytoplasm of podocytes individual tanks filled granular endoplasmic reticulum. There Golgi complex with small tanks, peroxisomes, individual polyribosomes. In a number of trabecular actin cytoskeleton is almost absent. The glomerulus is marked increase in the lumen of the capillaries and accumulation of erythrocytes and leukocytes.

CONCLUSION

Changes microscopic and submicroscopic structures surveyed bodies can be seen as destructive. Histological studies have shown that after the combined effects of dioxin in a dose 1/200 LD50, and lead acetate at a dose of 1/10 LD50 increased hepatic clearance between the beams. In some sinusoids detected cellular detritus, on the border small-globular precipitation. Hepatocytes are arranged randomly, lost the right radial topography hepatic beams. Cells acquire a more circular shape than in the control. In some hepatocytes observed irregular shape offset toward the periphery of the cytoplasm.

The cytoplasm vacuolated, with signs of granular dystrophy. The renal corpuscle is detected capillaries swelling and accumulation of blood cells. Podocytes with dense nuclei. Reduced clearance convoluted tubules. Logged granular dystrophy, which is characterized by impaired energy balance. The nuclei of some epithelial cells of the proximal and distal tubules become dense structure and become smaller. There are areas of desquamation of epithelial cells of the convoluted tubules.

The ultrastructure of mitochondria (increase in size, illumination, and matrix swelling) confirms granular dystrophy identified in histological images. In the cells of rabbit liver in combined toxicity of dioxin and lead acetate manifested pathological changes in the ultrastructure: violation of the TNG, vacuolization of cytoplasm, the destruction and fragmentation of the EPR. The rabbit kidney cells on the 40th day of combined poisoning with dioxin and lead acetate in doses of 1/200 and 1/10 LD50 violation occurs most ultrastructure, in varying degrees of changes of chromatin packaging, which leads to degradation of the nuclear apparatus.

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