

## Research Article

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## The peculiarities of ultra structural changes in kidneys in experimental intestinal ileus and in the reperfusion period

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### Abstract

In an experiment on rats we modeled the acute obstruction on the level of ileus. In 48 hours after the beginning of simulation of the indicated pathology we released the intestine from the gut. Using the electron microscopy we studied the peculiarities of changes in cell elements of the renal bodies that occur under the influence of intestinal obstruction, and after the restoration of patency of the small intestine. As a result, the research found that the progression of intestinal ileus in the haemomicrocirculatory bloodstream of kidneys appear the expressed discirculatory phenomena that lead to degenerative changes in endothelial cells of capillaries, and in epitheliocytes of tubules of the nephron. In cellular elements first appears the hyperhydration, which subsequently leads to the hydropic endothelial dystrophy in the endotheliocytes with the enlightenment of cytoplasm and the destructive changes in the organelles, picnosis of nuclei, chromatin margination. In the epithelial cells of the tubules of the nephron develops the destruction of cristae of mitochondria, the organelles themselves lose their orderly orientation, violates the density of intercellular contacts. The restoring of patency of the alimentary canal after its previous 48 -hour occlusion at first does not lead to improved blood circulation in the kidney and even, on the contrary, found previously disorders somewhat aggravate. The reason for this can be a "double blow" - local double hemodynamic and oxygen blow in the vessels of the small intestine. Further, there is a partial recovery of the structural elements of the kidney.

**Keywords:** Intestinal obstruction, Kidneys, Ultra structure, Reperfusion.

### Introduction

The critical post-operative states continue to be one of the difficult problems of modern surgery.<sup>1-3</sup> These include, first of all, mortality from multiple organ failure which remains at a high level.<sup>4, 5</sup> The frequent and often determining components of the multiple organ failure is the acute liver failure and kidney failure or hepatorenal syndrome.<sup>6</sup> It should be taken into account that a major pathogenetic factors of development of multiple organ failure include postischemic or reperfusion syndrome.<sup>7</sup> Ischemia-reperfusion often occurs in the treatment of the intestinal obstruction. The tissue hypoxia that develops at the same time just leads to the dysfunction of vital organs, mono-and multi-organ failure. Therefore, the study of the pathogenesis of tissue hypoxia and the search for new methods of its correction is one of the most fundamental and urgent problems of biology and medicine, and the development of new and effective methods of prevention will improve the outcomes, reduce disability and increase the life expectancy of patients.<sup>8</sup>

## Material and methods

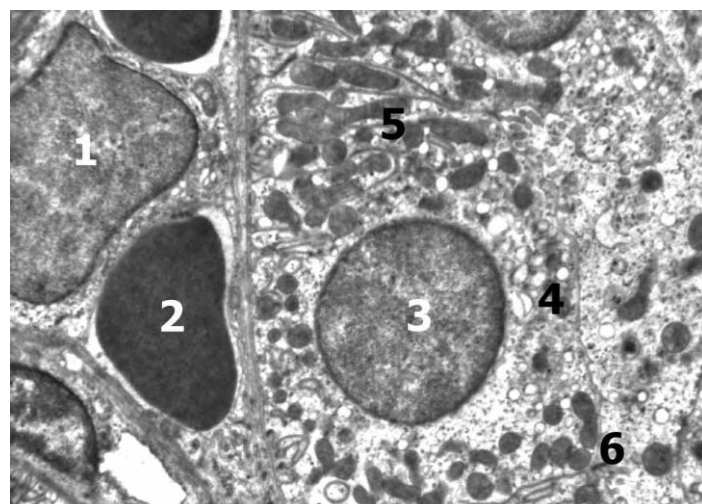
The experiments were performed on 30 white mongrel male laboratory rats, which were divided into three groups: one control group (6 animals) and two pilots (on 12 animals each). In the rats of the first experimental group we modeled the acute low obstructive ileus of the small intestine by means of weaving silk ligature 5 cm proximally the ileo-colic angle. For this purpose under a common intramuscular ketamine anesthesia median laparotomy was performed, the loop of intestine was taken out of the wound and through the hole in the mesentery between the arcades of the last order and the wall of intestine was carried out the ligature, which bypassed the intestine and tied it to the full coverage of the lumen. Laparotomy wound was sutured in layers. In the animals of the second experimental group at 48 hours after the initiation of the experiment we conducted relaparotomy, and then removed the ligature superimposed previously and reduced the intestine permeability. After the surgery, the animals were kept without food with plenty of water supply. The rats were derived of the experiment by the intrapleural injection of large doses of concentrated sodium thiopental. For the electron microscopic study of the excised kidney slices at 12, 24, 48 and 72 hours from the beginning of each phase of the experiment. All the experiments were performed in compliance with "The rules of work use the experimental animals," as confirmed by the Commission on bioethics of Ternopil State Medical University by I.Ya. Horbachevskyy (protocol number 11 from 15 April 2011).

## Results

The kidneys of intact rats have the typical ultrastructure, which is characteristic for other mammalian species. The nuclei are mostly oval shaped with the uneven terrain of kariolema due to the formation of invaginations. The slices of chromatin are scattered in carioplasma and concentrate near the inner nuclear membrane. The perinuclear space is narrow. In the close to the nucleus part of endotheliocytes the most of organelles are placed (elements of cytoplasmic granular mesh, Golgi apparatus, mitochondria, ribo- and polysomes, plasmolemal vesicles), whose number is relatively small. The endothelial cells are located on the basal membrane of uniform thickness. The close toothed shape contacts are formed between them. The glomerular capillaries are surrounded by podocytes. The podocytes consist of pericarion and processes that in radial way go to the periphery and which usually form a finite set of legs (pedicells) in contact with the basement membrane.

Between the closely intertwined legs of the adjacent podocytes remain the thin fissures of the filtration space. The tubular epithelial cells tight together and leaves no gaps between them. Nuclei of the round shape are filled with diffusely located euchromatin with small nucleoli. At their apical surface brush border is clearly differentiated.

After 12 hours from the start of simulation of the acute low intestine obstruction appear quite pronounced morphological changes in the cells of the structural elements of kidney. They were shown by the expansion of the lumen of blood capillaries of the peritubular grid. These microvessels were filled with blood corpuscles. The increasing of endothelial cells and their nuclei, as well as the expanding of the lumen of the tubular endoplasmic reticulum indicated on the active functioning of the haemomicrocirculatory channel. As a result, the cytoplasm of the endothelial cells seemed enlightened, sometimes with destructive changes of organelles. In its thinned sites were often manifested the different sizes fenestra.



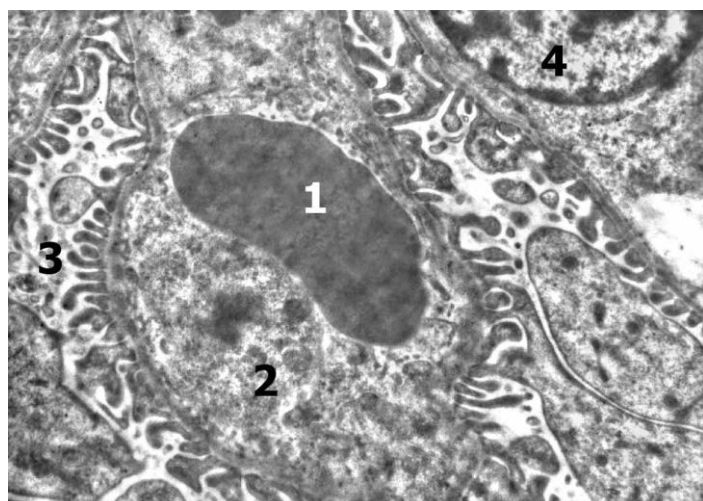
The core of endothelial cell of peritubular bloodcapillary of kidney - 1; erythrocyte in the lumen of the capillary - 2; core of the renal tubule epithelial cell - 3; expanding of tubular endoplasmic grid - 4 clusters of electronically tight mitochondria - 5; osmiophilic protein inclusions.

**Figure 1:** Electronogram of rat nephron items within 12 hours after onset of modeling of the acute high intestinal obstruction. x 18000

In epitheliocytes of proximal and distal nephron we observed the dystrophic phenomena, combined with the signs of increased functional load. In their enlightened by swelling cytoplasm was marked the decreased number of organelles. Expansion of tubules and cisterns of cytoplasmic reticulum and relatively high electron density of mitochondria showed some functional stress. The mitochondria often acquired the elongated shape. In

carioplasma of most nuclei contained a diffuse euchromatin, the perinuclear spaces were focally dilated. In many parts of the basal epithelial cells was marked the smoothing of membrane folds with the violation of ordering of location of mitochondria (Fig. 1). In epitheliocytes of proximal detected primary and secondary lysosomes and osmiophilic protein inclusions. Part of villus of their apical surface looked damaged.

In 24 hours from the beginning of monitoring the previously detected changes compounded. In the renal, glomerulus capillary cells markedly expanded the lumen of capillaries. The blood capillaries were mostly filled with red blood cells and other blood elements. It continued the enlightenment of endothelial cytoplasm by increasing of their hydration. The number of organelles in the cytoplasm decreased, and those that remained were often with the signs of degradation.

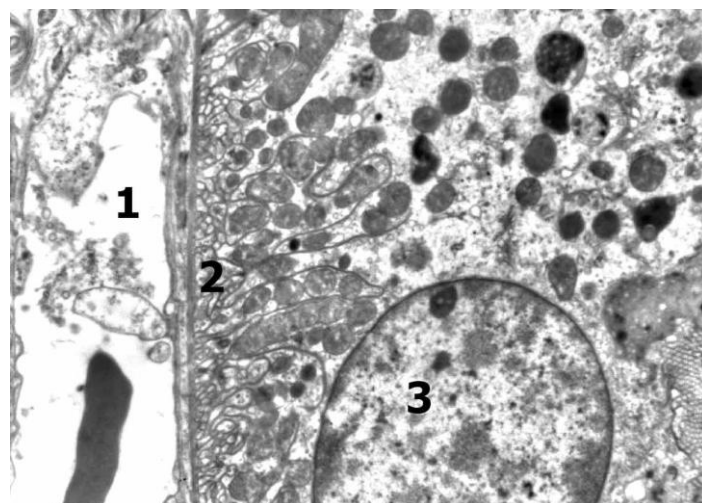


Red blood cell- 1; homogenized cytoplasm of podocyte- 2; numerous cytopodia- 3; nucleus of epithelial cell with heterologous chromatin - 4.

**Figure 2:** Electronogram of rat nephron items within 24 hours from the beginning of the simulation of acute high intestinal obstruction. x 18000

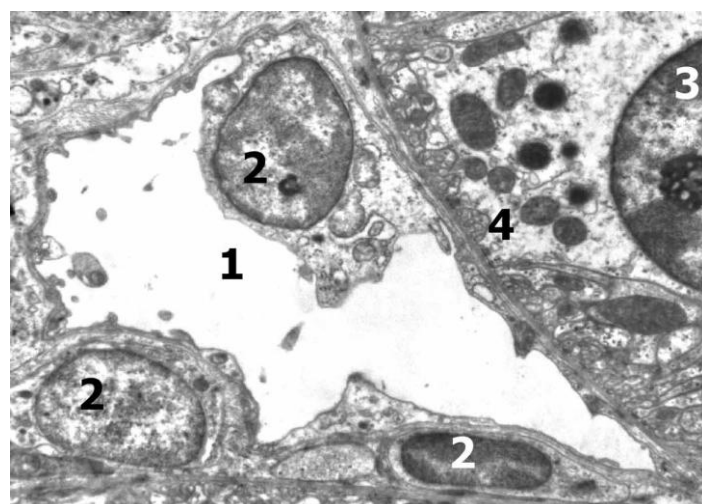
The cytolema of podocytes formed the numerous thinned cytopodia. The basement membrane weedered, and sometimes almost completely lost its structure (Fig. 2). The swelling of epithelial cells continued to grow with the enlightenment of the cytoplasm and an increase in the size of nuclei. In the nuclei accumulated and condensed the heterochromatin mainly localized close to the kariolema. In the cytoplasm of epithelial cells especially in the distal nephron in the subnuclear and basal areas appeared the different size vacuoles, in mitochondria the cristae destruction was recorded. The apical part at that time was

becoming particularly clear, swollen and lost organelles. There has been a violation of the integrity of the brush border (Fig. 3).



The lumen of the bloodcapillary- 1; vacuolization of basal parts of epithelial cell- 2; margination and condensation of heterochromatin in the enlarged core of epithelial cell of renal tubule- 3

**Figure 3:** Electronogram of rat nephron items within 24 hours from the beginning of the simulation of acute high intestine obstruction. x 18000



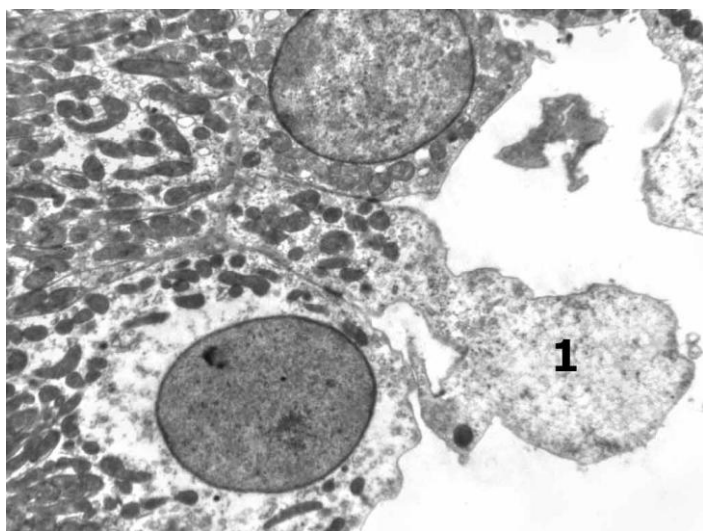
The lumen of the capillary- 1; nuclei of the epithelial cells of different shapes and sizes- 2; nucleus of epithelial cell- 3; violation of order of arrangement of mitochondria in the enlightened cytoplasm of endothelial cell - 4.

**Figure 4:** Electronogram of rat nephron items within 48 hours after the beginning of the simulation of acute high intestinal obstruction. x 18000

At the 48 hour duration of the experiment the phenomena of structural reorganization of cellular elements of the

nephron progressed. Renal glomerular capillaries cells continued to be extended and vibrant. The phenomenon of hydrotopic endothelial dystrophy was growing. The capillary lumen of the pretubular zone looked extended. The endothelial cells of this zone acquired various shapes and sizes. The condensation of heterochromatin and its periferisation occurred in nuclei. In the cytoplasm decreased the total number of organelles, were often observed the destruction of cristae in mitochondria. The basal membranes were uneven in thickness. The cytoplasm of epithelial cells of proximal tubules of the nephron in the nuclear-less areas enlightened, the number of mitochondria decreased and their arrangement became disordered (Fig. 4).

In the apical parts of the epithelial cells significantly disrupted the structure of microvilli of the border edging. It turned mitochondria in various stages of degradation and secondary lysosomes in the place of the damaged organelles. The nuclei of cells sometimes acquired the picnotic deformation due to the inequality and invaginations of kariolema. The destructive changes were found in epitheliocytes of the distal parts of tubules. The nuclei of these cells shifted to the apical pole and placed in a bright area of the cytoplasm. Often they lost the nucleus and detached of the basement membrane (Fig. 5).



Epithelial cell of the distal tubules of the nephron with the loss of core, destruction and homogenization of cytoplasmic organelles, that exfoliates into the lumen of the tubule- 1.

**Figure 5:** Electronogram of rat nephron items within 48 hours after the beginning of simulation of acute high intestinal obstruction. x 16000

The most intensive development of its destructive changes in cellular nephron cells acquired in the final stage of the experiment - 72 hours from the beginning. In the lumen of

blood capillaries as kidney cells and microvessels of the peritubular grid often were observed the elongated endothelial cells with flattened nucleus with chromatin margination. These endothelial cells hardly contained organelles, and the present mitochondria had the damaged cristae. As a result of the subendothelial swelling they detached from the picked basemnt membrane, violated the density of the intercellular contacts.

As to the epithelial cells, at the time of observation in the proximal nephron we revealed the so-called "dark" epithelial cells, which differed by the smaller sizes with more electronically desitive nuclei and cytoplasm which could be observed the destructive changes of organelles with their partial necrosis. The kariolema acquired a wavy look. Quite often could be observed the epithelial desquamation with the exfoliation of its cells in the lumen of tubules.

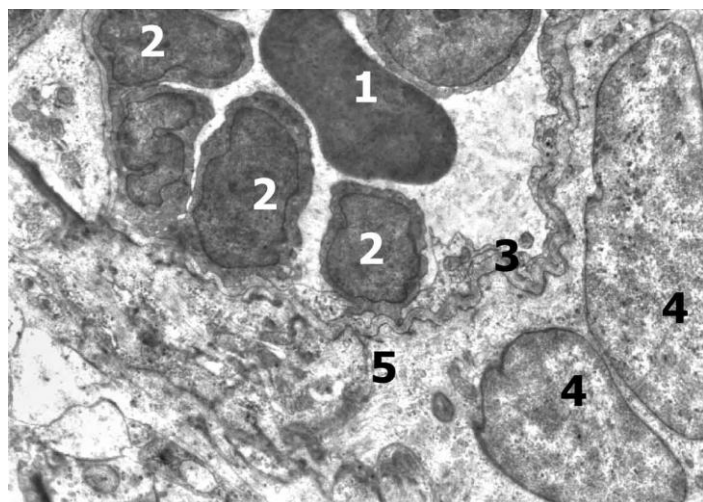
The strengthening of the dystrophic-destructive processes could be observed in the epithelium of the distal nephrons. They are manifested by the progressive enlightenment of cytoplasm, especially in the apical areas, in the basal parts of the cells were found the disordered mitochondria with homogenised osmiophillic matrix and the structure violation of membrane folds. It was also possible to find the migrating lymphocytes, which penetrated in the cytoplasm.

The results obtained in the course of further research, same as the results that have been obtained using the light-optical methods indicate that the restoration of patency of the alimentary canal since its previous 48-hour occlusion at first does not lead to the improved blood circulation in the kidney and even, on the contrary, earlier found disorders have aggravated.

However, during the first 12 hours of the reperfusion period any specific differences in the ultrastructural organization of cellular elements of nephrons compared with the 48-hour obstruction were not observed. Although in the vascular cells of the renal glomeruli revealed both a narrow blood capillaries and microvessels with the enlarged lumen. There has been a breach of the barrier wall seepage through basement membranes destructurization and expansion of fenestratsiy. The cytopodia of podocytes sometimes enhanced with injuries and the increase of distance between them. The cytoplasm of these cells electronically clear due to the swelling, it turned out relatively few organelles.

After 24 hours of reperfusion period the pathological changes grew and in their intensity markedly prevailed over the changes that were identified in the 48-hour simulation of acute high intestinal obstruction. Glomerular capillaries were significantly enhanced; their lumen was filled with the blood corpuscles, in the arteriolar departments often observed the aggregation of erythrocytes. It amplified the fenestration of the cytoplasmic areas of endothelium. The cytopodia of podocytes thinned, the areas of basement membranes bared, sometimes those were fragmented. The cytoplasm of cytotrabecula significantly enlightened.

In the venular parts of capillary were observed the phenomena of decrease of their bandwidth by the spatial reorientation of endothelial nuclei. The cytoplasm of the endothelial cells consolidated and the nuclei by their long axis were directed to the center of the lumen of the capillary, forming the picture of the "fence". The basal membrane thus acquired the winding paths. This was accompanied by the increasing of the destructive changes in the epithelial cells, the nucleus and cytoplasm of which enlighten and the number of organelles decreased (Fig. 6).



Erythrocytes in the narrowed lumen of the capillary- 1; vertical orientation of the condensed nuclei of endothelial cells in the form of "fence"- 2; folding of the basement membrane- 3; nuclei of epithelial cells- 4; enlightened parts of the basal cytoplasm of epithelial cells without organelles- 5.

**Figure 6:** Electronogram of rat nephron items in 24 hours after the reperfusion of the small intestine with the previous 48-hour simulation of acute high intestinal obstruction. x 16000.

In the next terms of the reperfusion period (after 48 and 72 hours from the beginning of the reperfusion of the small intestine), occurred the gradual recovery of the structural organization as haemomicrocirculatory route of nephrons and tubular epithelium. This was manifested by the

reducing of blood filling of capillaries, flattening of their endothelium, as well as the appearance and increasing of number of small-sized dense mitochondria with well-defined cristae in the basal parts of the epithelial cells and tubular endoplasmic grid. However, in the intercellular spaces increased the number of collagen structures. Though it should be noted that even after 72 hours of reperfusion period, full recovery has not yet occurred.

## Discussion

So the received in the process of research results indicate that the progression of intestinal obstruction in the renal blood circle arise discirculatory phenomena that lead to the dystrophic changes in endothelial cells of capillaries, and in epitheliocytes of the nephron tubules, which may be the result of the toxic effects from side of small intestine.<sup>9</sup>

The restoring patency of the alimentary canal after its previous 48-hour occlusion at first does not lead to improved blood circulation in the kidney and even, on the contrary, found previously disorders somewhat aggravate. The reason for this may be a "double blow" - local hemodynamic and oxygen blow in the vessels of the small intestine. In this case, changes in renal vessels arise secondarily, and perhaps already under the hemodynamic factors as well as additional toxic effects.<sup>10</sup>

## Conclusion

In the experimental intestinal obstruction as a result of toxic and hemodynamic effects in blood circulatory route of kidney arise the disorders that lead to the degenerative changes in the endothelial cells of capillaries and epitheliocytes cells of renal tubules.

The restoring of the contents passage along the small intestine at first does not lead to improvement of the morphofunctional state of the kidneys and on the contrary, the changes that occurred in the conditions of obstruction initially compounded due to the well-known dual hemodynamic oxygen blow.

Further, there is a partial recovery of the structural elements of the kidney. However, the full recovery does not occur - which may be the cause of the progression of sclerotic changes that occur under the conditions of prolonged hypoxia.

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