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PECULIARITIES OF MYOCARDIAL ULTRASTRUCTURE OF RATS AT THE LATE TERMS OF OPIOID INTOXICATION

Abstract. *In recent decades drug addiction in Ukraine has spread very quickly. In 2015 the WHO estimated approximately 400 000 injection drug users in Ukraine. Every year the number of drug addicts in Ukraine becomes 8-9% more. A negative tendency is the fact that 70-75% of drug addicts are young people under 25 years of age. A considerable use of narcotic substances, their increased circulation and spread of drug addiction stipulate the necessity of detailed studies of opioid effect on the human organism, and morphological structure of the internal organs in particular. The majority of conclusions are based on clinical administration of drugs without adequate experimental studies concerning morphological changes, which is intolerable. Therefore, the objective of our study is investigation of opioid effect on the human organism and morphological structure of the internal organs in particular, determine and describe morphological changes in the myocardium of rats and its blood microcirculation on the ultra-structural level under Nalbuphine effect on the 28th and 42nd days of experimental opioid intoxication. The study was conducted on 48 laboratory mature albino male rats with the body weight of 130-200g, aged from 3,5 to 4,5 months. The animals were divided into two groups – experimental and control. The experimental animals were every day intramuscularly injected with the opioid Nalbuphine (nalbuphine hydrochloride) produced by Rusan Pharma with increasing the dose from 25 mg/kg to 35 mg/kg according to the following plan: I week – 8 mg/kg, II week – 15 mg/kg, III week – 20 mg/kg, IV week – 25 mg/kg, V week – 30 mg/kg, VI week – 35 mg/kg. The rats from the control group were intramuscularly injected with sodium chloride. Heart samples were used as the material for investigation. The material for morphological examination was taken under control of biochemical blood indices (LPO, superoxide dismutase, catalase, malonic dialdehyde, glutathione peroxidase, glutathione transferase). The method of electron microscopy of samples was applied. The material was examined and photos taken by means of the microscope УЕМВ–100К (Ukraine) with accelerating potential 75kW and 4000 – 24000 x magnification on the microscope screen. The results are indicative of the fact that against the ground of injection of the opioid analgesic Nalbuphine on the 28th day of the experimental opioid intoxication deep destructive changes of cardiomyocytes similar to those of myocardial infarction are found: destruction of cardiomyocytes, mitochondrial crystallolysis, intercellular swelling, sludge syndrome and cellular detritus available in the vascular lumen. On the 42nd day of the experimental opioid intoxication the signs of decompensation and destruction of cardiomyocytes increase, the perivascular space dilates due to swelling and cellular detritus blocking the vascular lumen, changed erythrocytes and formation of clots. Villous damage of the cardiomyocyte sarcolemma is characteristic for this term of the experiment.*

Key words: *opioid, Nalbuphine, myocardium, blood microcirculation, rat.*

Introduction. In recent decades drug addiction in Ukraine has spread very quickly. In 2015 the WHO estimated approximately 400 000 injection drug users in Ukraine. Every year the number of drug addicts in Ukraine becomes 8-9% more. A negative tendency is the fact that 70-75% of drug addicts are young people under 25 years of age. Gender analysis is indicative of the fact that boys use drugs more often than girls. 73% of drug users are urban residents, although a part of the rural youth increases gradually. 97% drug addicts tried drugs for the first time at the age of 12-19, and every fifth drug addict is a female [10]. Therefore, a considerable use of narcotic substances, their increased circulation and spread of drug addiction stipulate the necessity of detailed studies of opioid effect on the human organism, and morphological structure of the internal organs in particular.

The organs affected by narcotic substances

microscopically resemble clinical signs peculiar for chronic inflammatory diseases. At the same time internal organs are filled with blood irregularly, the majority of the blood vessels are spasmodic, and blood in them is mostly watery, dark, with single loose clots [7].

Acute poisoning with narcotic substances is characterized by hyperemia of the cerebral tunics and hemorrhages into its matter [4]. Deep destructive changes of nerve cells, their organelles, cytoplasm blooming, formation of vacuoles, and development of microangiopathy are found [1].

In addition to swelling of the drainage glia manifested by perivascular and pericellular swelling microcirculatory disorders in the form of erythrocyte stasis in the capillaries, general venous plethora, and erythrocyte sludge are found. Sometimes hemorrhages involve pia mater [3].

Microscopic examination of the adrenal glands detects areas with lost structure of the glomerular zone, absence of the borders between the cortical zones, and focal delipoidization. Hemodynamic disorders are characterized by plethora, signs of erythrocyte stasis, formation of sludge and clots. In addition, pyknotic dense nuclei of chromaffin and supporting cells, and cytoplasm vacuolization are found [2]. Morphological examination of the kidneys after opioid intoxication detects vascular reactions of the glomeruli in the sub-capsular and juxtacellular areas of the renal cortex in the form of hypercellularity, dilation and overfilling of the capillary loops with erythrocytes, dilation of the mesangial matrix of the renal glomeruli. In addition, thickening of the collagen fibers of the external layer of the glomerular capsule and lymphohistiocytic infiltration of the periglomerular stroma are found [5]. Massive swelling in the lungs is detected by macroscopic examination. Vascular plethora with the signs of stasis and swelling are found on histological specimens. Emphysema areas are found in the lung parenchyma interchanging with atelectasis areas. The groups of swollen alveoli with admixtures of neutrophil leukocytes are found rather frequently. A number of alveolocytes and macrophages were found in these areas which was indicative of availability of small pneumonic foci in the lungs [6].

The liver is characterized by the signs of fatty degeneration and stasis plethora of the portal tracts. Periportal cellular inflammatory infiltration and dystrophic changes of the liver cells up to focal necrosis were found on histological examinations [7]. The liver cells have dystrophic changes manifested by moderate swelling of the liver cells with granulation in their cytoplasm which is indicative of granular dystrophy of the liver cells. The nuclei of the cells are of different sizes. In many cases small and moderate fatty drops were found located in the center of the lobes which is indicative of fatty degeneration [6]. Nalbuphine intoxication of the liver is characterized by changes of the blood microcirculation in the form of dilation or narrowing of sinusoids, hemostasis with formation of clots and hemorrhages, and lymphohistiocytic infiltration, focal necrosis and increased amount of apoptotic changed cells prevail [8].

Many researchers describe morphological changes of the heart of drug addicts both on the micro- and ultra-structural levels, although pathogenesis of opioid effect on the myocardium and its blood microcirculation, and what morphological changes are caused by opioids still remain unknown. The majority of conclusions are based on clinical administration of drugs without adequate experimental studies concerning morphological changes, which is intolerable.

Objective: to determine and describe morphological changes in the myocardium of rats and its blood microcirculation on the ultra-structural level under Nalbuphine effect on the 28th and 42nd days of experimental opioid intoxication.

Materials and methods. The study was conducted on 48 laboratory mature albino male rats with the body weight of 130-200g, aged from 3,5 to 4,5 months. The animals were carefully selected for the study. Every rat was examined, weighed, and marked. The selected group of animals was kept in a separate cage on standard food in the vivarium. A careful examination prevented to involve the animals with signs of intra-vivarium infection into the groups of control and experiment. The animals were divided into two groups – experimental and control. The experimental animals were every day intramuscularly injected with the opioid Nalbuphine (nalbuphine hydrochloride) produced by Rusan Pharma with increasing the dose from 25 mg/kg to 35 mg/kg according to the following plan: I week – 8 mg/kg, II week – 15 mg/kg, III week – 20 mg/kg, IV week – 25 mg/kg**,** V week –

30 mg/kg, VI week – 35 mg/kg [9]. The rats from the control group were intramuscularly injected with sodium chloride. Heart samples were used as the material for investigation. The material for morphological examination was taken under control of biochemical blood indices (LPO, superoxide dismutase, catalase, malonic dialdehyde, glutathione peroxidase, glutathione transferase). The material for the study was taken every week by means of exsanguination of animals after intraperitoneal injection of sodium thiopental. All the investigations were carried out according to the regulations of the Directive of the European Commission dated Novenber 24, 1986, and were approved by the Bioethical Committee at Danylo Halytskyi Lviv National Medical University, the minutes №3 dated March 16, 2015.

To obtain ultra-thin sections the fragments of the rats' hearts were cut by means of a blade, and placed immediately into a large drop of 2% osmium tetroxide solution on 0,1 M phosphate buffer (pH 7,36) with sucrose. After that the strips of the cardiac tissue 0,8×0,1×0,1cm in size were cut by means of a blade degreased in acetone, and they were quickly placed into another drop of a fixing solution of the same content located on the plate of dental wax lying on an ice slab. Small pieces (tissue blocks) of the cardiac tissue, cubic in shape and $1mm^3$ in volume were cut from the strips. The tissue blocks were fixed in 2% osmium tetroxide solution on 0,1 M phosphate buffer (pH 7,36) with sucrose addition during 2 hours. After that they were washed by buffer solution of the same content (4 fresh portions 15 minutes in each). The material was conducted according to the standard scheme [11]. Ultra-thin sections were prepared on the ultramicrotome УМТП-3М by means of glass knives prepared on the device ССН-1. The sections of a silver or delicately lemon colour were selected for the study. First the sections were contrasted in 2% uranyl acetate solution, followed by lead citrate [12]. The material was examined and photos taken by means of the microscope УЕМВ–100К (Ukraine) with accelerating potential 75kW and 4000 – 24000 x magnification on the microscope screen.

Results and discussion. Ultramicroscopic examination of rats intramuscularly injected with the opioid Nalbuphine during 4 weeks of the experiment on the 28^{th} day detected that sarcolemma of the cardiomyocytes was thickened, and destructed in some areas. Mitochondria are broken, glycogen granules are absent. The sarcolemma of cardiomyocytes is destroyed with invagination areas. Mitochondria destroyed and changed by their shape and size are located marginally. Myofibrils are broken. Intracellular swelling is found (Fig.1) and myofibrils, Z-disks and M-lines are ruined. T-system is dilated, destructed, with visible lysosomes.

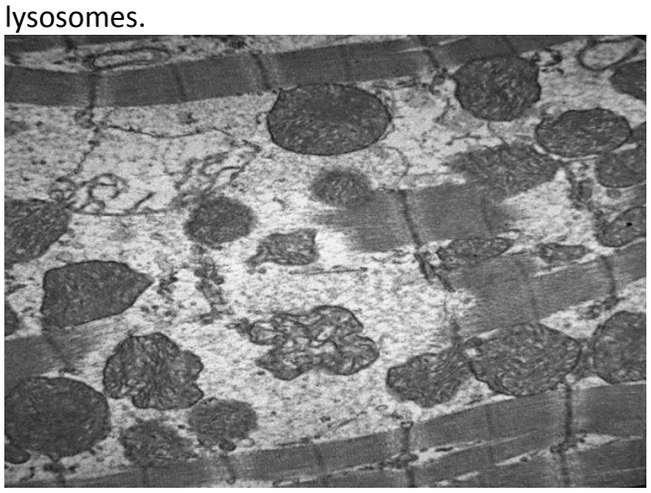

Fig.1. Intracellular swelling in the myocardium in 28 days of the experiment. Electronic micrograph. Magnification : ×10000

Many broken mitochondria are found. Preserved mitochondria are changed by their size and shape, and their mitochondrial matrix is compact. Sarcoplasmic reticulum is dilated and partially lysed. In some areas myofibrils are destructed, Z-lines, M-lines are ruptured, and mitochondria are changed by their shape and size. A considerable damage of the sarcoplasmic reticulum is found between myofibrils. Glycogen blobs are absent which is indicative of decompensation processes in the myocardial cells (Fig.2).

Although at this term of the experiment Z-disks and M-lines are preserved in certain areas of cardiomyocytes (Fig.3).

Marginal changes of cardiomyocyte sarcolemma, destruction of myofibrils, thickening of the contacts between cardiomyocytes were found in the experimental animals. Chromatin is marginally located in the fibroblast nucleus. The folds of the cytoplasmic membrane are found (Fig.4).

In the area of histohaematin barrier blood capillaries adhere closely to the surrounding cardiomyocytes. Invaginations and expansions are found on the luminal surface of the endothelial cells. Erythrocytes are changed in the lumen of blood capillaries by their shape and size, platelets

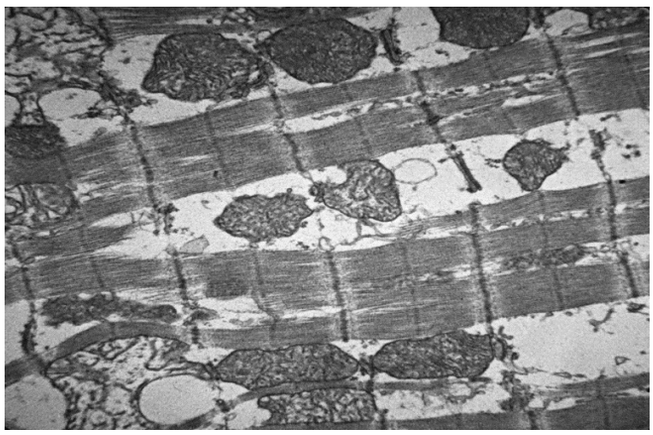

Fig. 2. Central part of cardiomyocyte of a rat 28 days after the experiment. Electronic micrograph. Magnification: x 10 000.

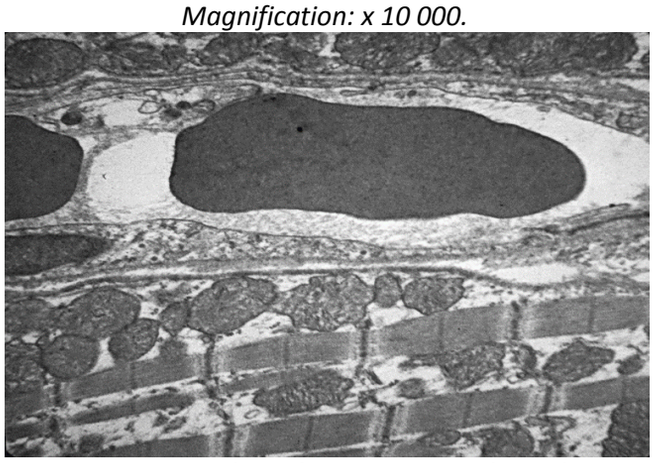

Fig.3. Marginal fold of the luminal surface of the endotheliocyte. Electronic micrograph. Magnification : x 5000

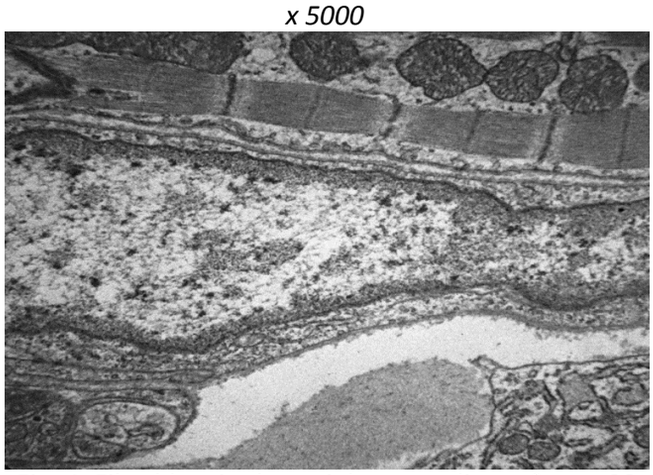

Fig. 4. Fibroblast in the atrial myocardium of a rat 28 days after the experiment. Electronic micrograph. Magnification : x 10 000.

adhesive to the luminal membrane of the endotheliocytes are found between them. The basal membrane of the majority of capillaries is laminated and destructed in some areas. The number of pinocytic vesicles in endotheliocytes at this term is not changed.

At this term of the experiment the lumen of microvessels is filled with cellular detritus (Fig.5). The luminal surface of the endotheliocytes is changed which is presented by numerous folds and invaginations. Numerous pinocytic vesicles and destruction of mitochondrial apparatus are available in the cytoplasm of endotheliocytes. Cellular detritus is found in the lumen of blood capillaries. Pathological folds are found on the luminal surface of endotheliocytes which are 3-4 times as much as marginal ones. Pinocytic vesicles and lysosomes are found in the cytoplasm of endotheliocytes.

On the 42nd day of the experiment a dilated intercellular space is found due to swelling, deformation of sarcolemma, swelling with destruction of sarcoplasmic reticulum is found between myofibril fibers, the majority of mitochondria are destructed, although some of them are preserved (Fig.6).

Vacuolization and mitochondrial crystallolysis are detected between myofibrils and under sarcolemma (Fig.7).

Mosaic damage of the surrounding cardio-myocytes is found in the areas of the central parts of cardiomyocytes where practically undamaged cardiomyocyte is located close to the damaged

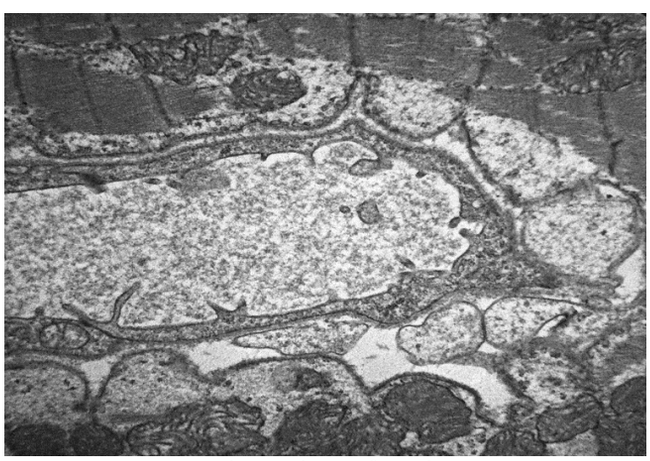

Fig. 5. Venule 28 days after the experiment. Vascular lumen is filled with cellular detritus. Electronic micrograph. Magnification : × 8000.

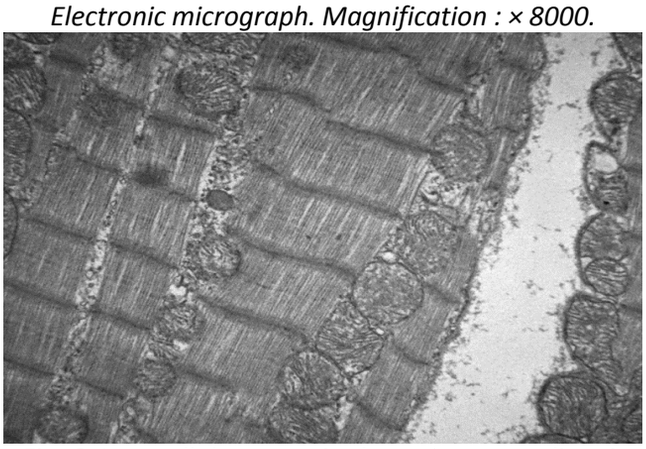

Fig. 6. Intercellular space in the rat's myocardium is dilated at the expense of swelling 42 days after the experiment. Electronic micrograph. Magnification : x 5000.

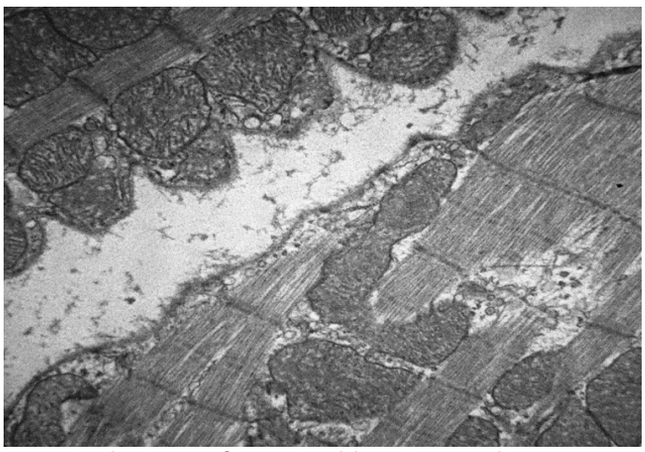

Fig. 7. The area of two neighbouring cardiomyocytes 42 days after the experiment. Vacuolization and crystallolysis of mitochondria are seen between myofibrils and under sarcolemma. Electronic micrograph. Magnification : x 5000.

one. Exfoliation of myofibrils, destruction of Z-disks and M-lines with simultaneous preservation of these structures in the neighbouring myofibrils, destruction of mitochondrial crests, and dilation of T-system canals are found (Fig. 8).

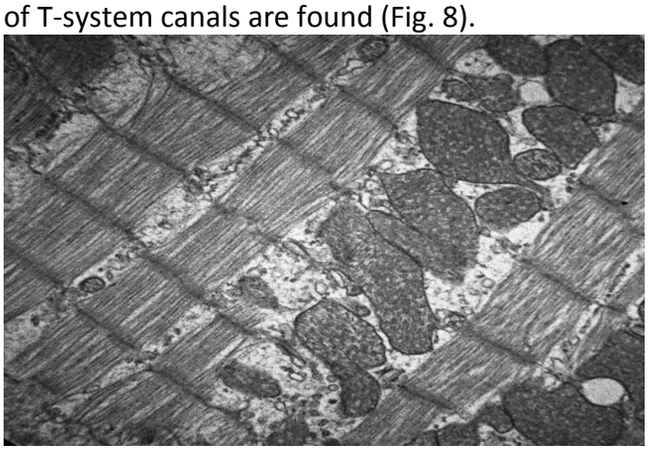

Fig.8. Area of the central part of the cardiomyocyte of a rat 42 days after the experiment Electronic micrograph. Magnification : x 5000.

A considerable perivascular swelling in the area of histohaematic barrier, ruined sarcolemma of cardiomyocytes, ruined Z-disks between myofibrils are determined. Sarcolemma of cerdiomyocytes has folds with the signs of swelling, glycogen blobs are absent, mitochondrial crests are ruined. Myofibrilolysis of cardiomyocytes and dilation of T-system tubes are detected (Fig.9).

Villous damage of the cardiomyocyte sarcolemma is specific for this term of the experiment (Fig.10).

Blood capillaries with thickened fold membrane and cellular detritus in their lumen are found (Fig.11).

The vascular wall is swollen and exfoliated, the internal layer of membranes has folds and finger-like expansions. Interendothelial contacts are thickened. Smooth muscular contacts are damaged. Cellular detritus of formed blood elements is found in the vascular lumen (Fig.12).

In the area of histohaematic barrier a considerable dilation of the interstitial space, swelling and partial destruction of the internal layer of the membrane are detected. The vascular

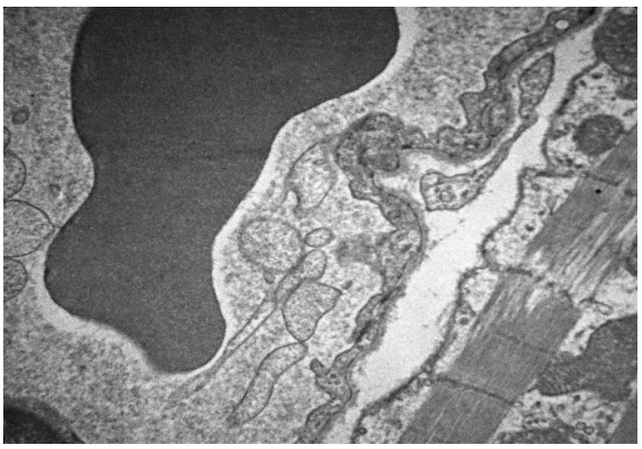

Fig. 9. Area of histohaematic barrier of the rat's myocardium 42 days after the experiment Electronic micrograph. Magnification : x 5000.

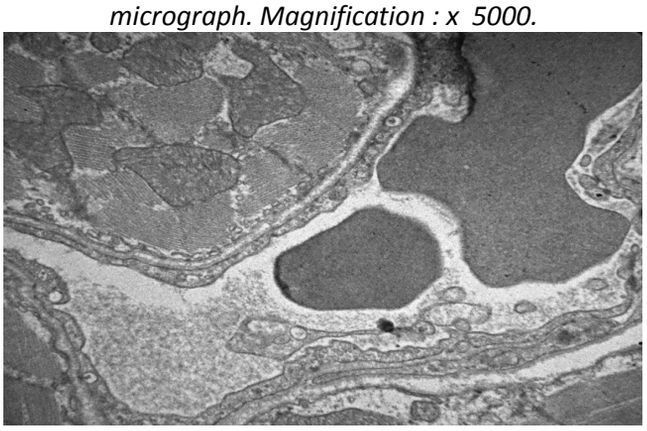

Fig. 10. Villous damage of the cardiomyocyte sarcolemma of a rat 42 days after the experiment. Fragments of platelets, erythrocytes changed by their shape and size are found in the vascular lumen. Electronic micrograph. Magnification : x 5000.

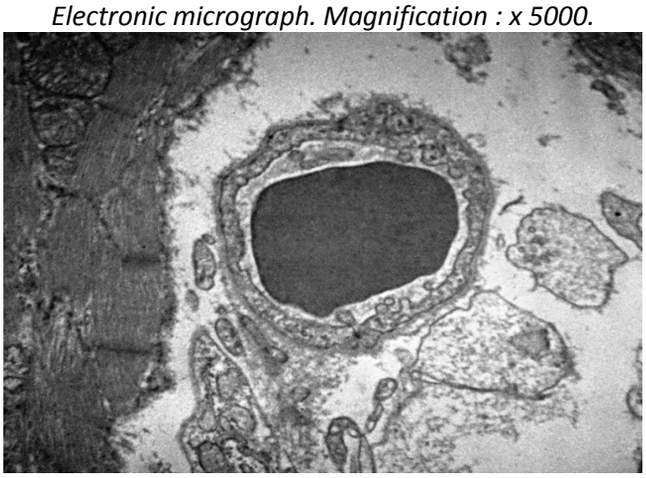

Fig. 11. Area of histohaematic barrier of the rat's myocardium 42 days after the experiment Electronic micrograph. Magnification : x 5000.

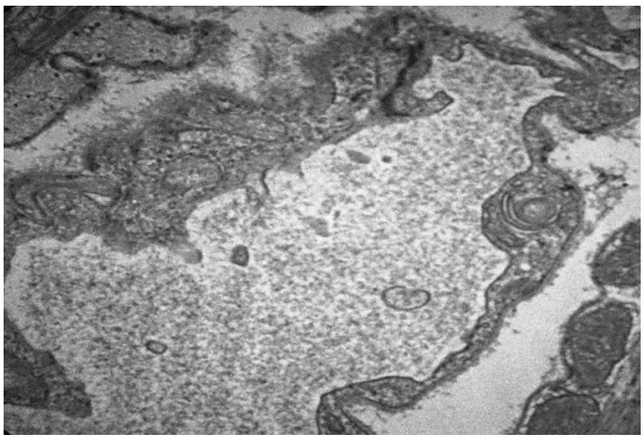

Fig. 12. Area of histohaematic barrier of the rat's myocardium 42 days after opioid intoxication. Electronic micrograph. Magnification : x 5000.

lumen is filled with changed erythrocytes and remains of the formed blood elements and cellular detritus.

Conclusion. On the 28th day of the experimental opioid intoxication deep destructive changes of cardiomyocytes similar to those of myocardial infarction are found: destruction of cardiomyocytes, mitochondrial crystallolysis, intercellular swelling, sludge syndrome and cellular detritus available in the vascular lumen.

On the 42nd day of the experimental opioid intoxication the signs of decompensation and destruction of cardiomyocytes increase, the perivascular space dilates due to swelling and cellular detritus blocking the vascular lumen, changed erythrocytes and formation of clots. Villous damage of the cardiomyocyte sarcolemma is specific for this term of the experiment.

Prospects of further studies. The obtained results enable to extend the notion and solve the issue concerning opioid effect on the heart structure and its blood microcirculation forming morphological basis for better understanding of pathogenesis and further search of optimal methods of treatment of cardiologic diseases among patients who have to take opioids for a long period as well as drug addicts.

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