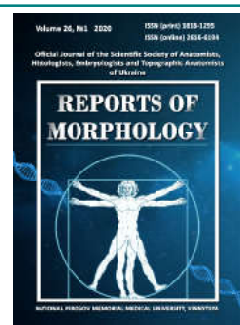




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# Submicroscopic changes in the liver of young rats with hyperhomocysteinemia

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*To date, it has been established that hyperhomocysteinemia plays a significant role in the development and progression of many diseases. The accumulation of homocysteine occurs due to a violation of the relationship between its production and excretion from the body. The liver plays an important role in the metabolism of homocysteine, because it undergoes most of the reactions of its transmethylation, and, therefore, it is the first to be adversely affected. The aim of the study is to identify the features of electron microscopic changes in the liver structure of young rats with hyperhomocysteinemia. The experimental study was performed on 22 white nonlinear young (1-2 months) male rats, which were divided into a control group and an experimental group. A model of persistent hyperhomocysteinemia was created by administering to rats the experimental group of thiolactone homocysteine at a dose of 200 mg/kg body weight intragastrically for 60 days. The study of ultrastructural changes in the liver of rats was performed using an electron microscope PEM-125K. It was found that the introduction of thiolactone homocysteine at a dose of 200 mg/kg in rats led to the development of degenerative changes in hepatocytes. Changes in the structure of liver cells manifested themselves in the form of edema of the cytoplasm and mitochondria, destruction of mitochondrial cristae, dilation of the tubules of the granular endoplasmic reticulum and tanks of the Golgi complex. The activity of fat-accumulating liver cells and stellate macrophages is characteristically. In the lumens of the sinusoidal capillaries found sweeter shaped blood elements, the cytoplasm of endothelial cells had signs of edema. Thus, in experimental hyperhomocysteinemia revealed changes at the ultrastructural level in all structural components of the liver of young rats. The identified changes are compensatory-adaptive in nature and are reversible.*

**Keywords:** hyperhomocysteinemia, hepatocytes, mitochondria, macrophages, sinusoidal capillaries.

## Introduction

In the last few years, the amino acid homocysteine has attracted a lot of attention from researchers. This phenomenon is associated with the emergence of new research, which shows the extremely negative impact of elevated levels on the body and participation in the pathogenesis of many diseases. Homocysteine is a non-protein sulphhydryl amino acid formed during the metabolism of the essential amino acid methionine [16, 18, 19]. Intracellular homocysteine metabolism is carried out in several stages. S-adenosylmethionine is synthesized from methionine with the participation of the enzyme methionine adenosyltransferase. The latter, losing the methyl group, is converted to S-adenosylhomocysteine. Subsequently, adenosine and homocysteine are formed due to S-

adenosylhomocysteine hydrolase. The process of elimination of excess homocysteine in the body is provided by three ways - remethylation, transsulfation, desulfurization [9, 15]. The mechanism of remethylation is based on the process of resynthesis of methionine from homocysteine with the obligatory participation of vitamin B12 and methionine synthase. In the process of transsulfation, homocysteine is converted to cystathionine under the action of the enzyme cystathionine- $\beta$ -synthetase. Subsequently, the formed cystathionine, already under the influence of vitamin B6-dependent enzyme  $\gamma$ -cystathionase decomposes into cysteine, ammonia and  $\alpha$ -ketobutyrate [7]. Due to the transsulfation process, the excretion of up to 70-80% of excess homocysteine levels is ensured, the rest is

accounted for by remethylation and desulfation mechanisms. As a result of the latter, homocysteine is converted to hydrogen sulfide.

Many studies have been assigned to the study of the pathogenetic effects of elevated plasma homocysteine levels, but to this day this issue remains incomplete. Most researchers identify several mechanisms of adverse effects of hyperhomocysteinemia. These include: activation of oxidative stress, expression of mediators of inflammation and fibrosis, inhibition of methylation processes, homocysteine protein synthesis [9, 14, 16].

Scientists around the world have begun to actively study the effects of hyperhomocysteinemia on the structure and function of the liver. Being an important organ for life, it takes an active part in the processes of metabolism and utilization of homocysteine, which is due to the considerable interest of researchers. The pathogenesis of liver cell damage today remains poorly understood. It is established that the damage is based on endothelial dysfunction caused by hyperhomocysteinemia, lipid and protein peroxidation. There are also hypotheses about the direct toxic effects of homocysteine on the mitochondria of hepatocytes [4, 8].

Thus, the study of the features of electron microscopic changes in the structure of the liver is an important and urgent task that will expand the understanding of the pathogenesis of its lesions in hyperhomocysteinemia.

The aim of the study is to identify the features of electron microscopic changes in the liver structure of young rats with hyperhomocysteinemia.

### Materials and methods

The experiments were performed on 22 white nonlinear young (1-2 months) male rats, which were divided into a control group and an experimental group. A model of persistent hyperhomocysteinemia was created by administering to rats the experimental group of thiolactone homocysteine at a dose of 200 mg/kg body weight intragastrically for 60 days. Animals were decontaminated by decapitation under thiopental anesthesia. Committee on Bioethics of National Pirogov Memorial Medical University, Vinnytsya found that the experimental study was conducted considering the recommendations of the European Commission for medical and biological research using animals, medical recommendations of the State Pharmacological Center of the Ministry of Health of Ukraine and "Rules for clinical safety assessment of pharmacological agents (GLP)".

For morphological examination, pieces of liver 0.5-1.0 mm in size were taken and fixed in a 2.5% solution of glutaraldehyde with an active reaction medium pH 7.2-7.4, prepared on phosphate buffer. Next, the material was postfixed in a 1% solution of osmium tetroxide according to Caulfield. Dehydrated in alcohols of increasing concentration (70%, 80%, 90%, 100%) and acetone [6]. Poured into a mixture of epon-araldite. Semi-thin sections were made from the obtained blocks, which were stained with toluidine blue

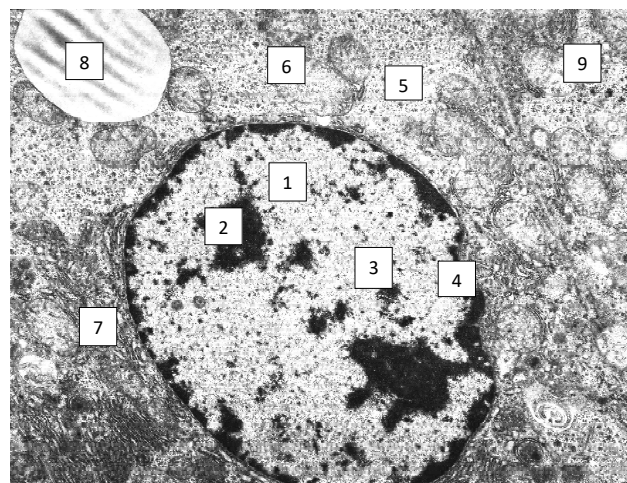
and Hyatt. After aiming at semi-thin sections, ultrathin sections contrast with 2% uranyl acetate solution and lead citrate were made on LKB III (Sweden) and Reihart (Austria) ultratomas. The preparations were examined and photographed under an electron microscope PEM-125K.

### Results

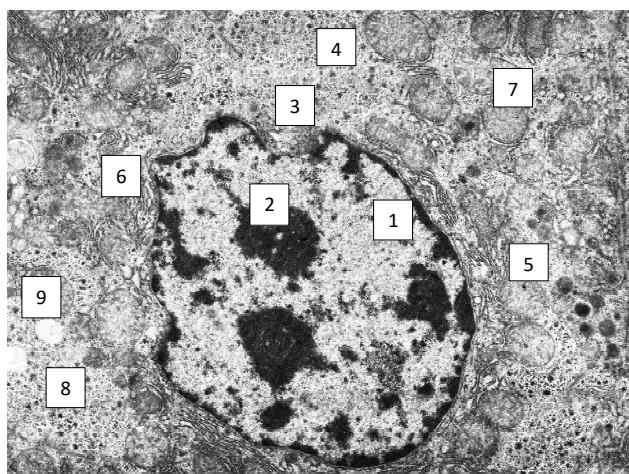
Electron microscopic studies of the liver of young rats under conditions of hyperhomocysteinemia caused by the introduction of thiolactone homocysteine at a dose of 200 mg/kg, found the presence of moderate dystrophic changes in hepatocytes. The latter manifested as accumulations of heterochromatin in the marginal and central parts of the nucleus. The nuclear membrane was characterized by the presence of intussusception, compared with the control group (Fig. 1).

In the cytoplasm of hepatocytes there is a significant number of mitochondria, the size of which is larger than that of intact young rats. They are characterized by the presence of a fine-grained matrix and numerous cristae. It should be noted that in some places hepatocytes were found, in which mitochondria formed buds, were swollen, with signs of disorganization and destruction of the cristae. The cisterns of the granular endoplasmic reticulum are dilated, and a large number of ribosomes are present on its membranes. Hyperplasia of the membranes of the endoplasmic reticulum was detected in some hepatocytes. Their cytoplasm contained numerous ribosomes, polysomes and granules of glycogen (Fig. 2).

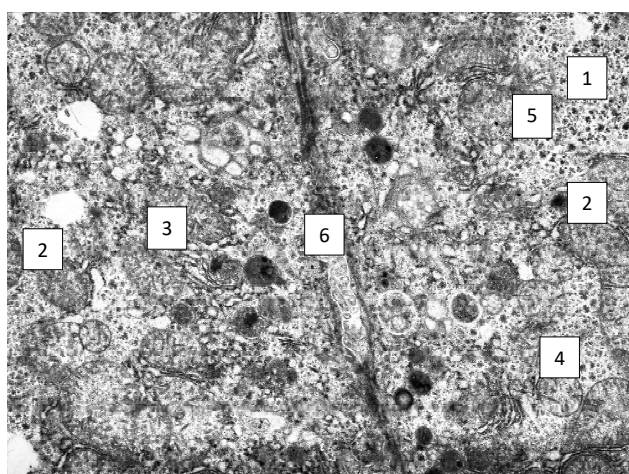
Cells with hypertrophied Golgi complex and high content of autophagosome and fat droplets were also detected. The lumens of the bile ducts are dilated, they have few microvilli. However, the destruction of hepatocyte microvilli in the bile capillaries and perisinusoidal spaces was not detected (Fig. 3).



**Fig. 1.** Electronogram of the liver of young rats with hyperhomocysteinemia: 1 - hepatocyte nucleus, 2 - nucleolus, 3 - clumps of heterochromatin, 4 - intussusception of karyolemma, 5 - cytoplasm of hepatocyte, 6 - destruction of cristae and edema of the matrix of mitochondria, 7 - tanks of granular endoplasmic reticulum, 8 - fat droplets, 9 - glycogen granules. x4800.

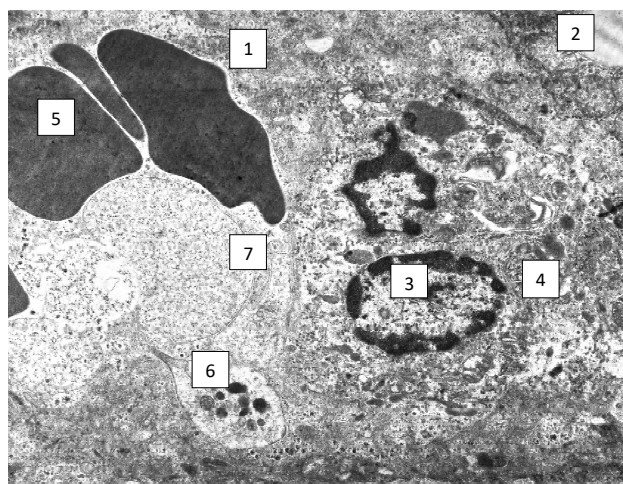


**Fig. 2.** Electronogram of the liver of young rats with hyperhomocysteinemia: 1 - hepatocyte nucleus, 2 - nucleolus, 3 - karyolemma intussusception, 4 - hepatocyte cytoplasm, 5 - mitochondria, 6 - granular endoplasmic reticulum tanks, 7 - lysosomes, 8 - the inclusion of glycogen, 9 - fat droplets. x4800.



**Fig. 3.** Electronogram of the liver of a young rat with hyperhomocysteinemia: 1 - hepatocyte cytoplasm, 2 - mitochondria, 3 - granular endoplasmic reticulum tanks, 4 - lysosomes, 5 - glycogen lumps, 6 - bile duct lumen. x6400.

The nuclear envelope of endothelial cells in the walls of sinusoidal capillaries was moderately fluffy, had different depths of intussusception. In the cytoplasm of endotheliocytes, a small number of swollen mitochondria and electron-transparent tanks of the granular endoplasmic reticulum were found. It should be noted the increased number of cristae in mitochondria, as well as ribosomes on the membranes of the granular endoplasmic reticulum compared to those in intact young rats. Increased number of freely located in the cytoplasm of secondary lysosomes. The plasma membrane of endothelial cells formed numerous outgrowths in the lumen of the sinusoids. In the cytoplasm of stellate macrophages there are significantly more heterophagosomes and autophagosomes than in intact young rats, which indicates increased functional activity of these cells (Fig. 4).



**Fig. 4.** Electronogram of the liver of young rat with hyperhomocysteinemia: 1 - vascular part of the cytoplasm of the hepatocyte, 2 - a drop of fat, 3 - the nucleus of the stellate macrophagocyte, 4 - the cytoplasm of the stellate macrophagocyte, 5 - sludge of erythrocytes in the lumen of sinusoid, 6 - platelets in the lumen of the sinusoid, 7 - swelling of the cytoplasm of the endothelial cell in the sinusoidal wall. x4800.

### Discussion

The results of electron microscopic examination of the liver structure under conditions of hyperhomocysteinemia are consistent with the available data in the literature. It is established that hyperhomocysteinemia causes enhancement of biosynthetic processes in hepatocytes and is characterized by the development of dystrophic changes in them. The latter will cover in most cases the nuclear apparatus and mitochondria. Hyperhomocysteinemia also causes the appearance of so-called mitochondrial spheroids and concentric orientation of granular endoplasmic reticulum tanks. In addition, there is damage to the microcirculatory tract of the liver and the development of fibrogenesis [12].

High concentrations of homocysteine in blood plasma are associated with the development of hydropic and fatty liver disease. In addition, histological examination also reveals histio-leukocyte infiltration of the portal areas, hyperplasia of stellate cells, necrosis of hepatocytes, fibrosis in the portal areas and around the central veins [3, 11].

Hyperhomocysteinemia leads to increased energy metabolism in the mitochondria of hepatocytes, which is manifested by increased activity of lactate dehydrogenase, succinate dehydrogenase and H<sup>+</sup>-ATPase. This condition is characterized by impaired calcium deposition, increased carbonylation of mitochondrial proteins, a significant decrease in reserve-adaptation potential and increased superoxide dismutase activity [10, 20].

It is established that one of the reasons for the development and progression of non-alcoholic fatty liver disease may be high levels of homocysteine in the blood. Hyperhomocysteinemia is the basis for the occurrence of steatohepatitis, an increase in the lipid spectrum of the blood, and subsequently - the growth of the process into

fibrosis and cirrhosis of the liver [1, 2]. The development of fibrosis is more likely to occur due to inhibition of hepatocyte regeneration and increased proliferation of fibroblasts [5].

There are data on the negative effects of homocysteine on protein, carbohydrate and fat metabolism in liver cells [21]. At the optical level, these disorders are manifested in the form of steatosis, multilobular fibrosis with signs of parenchymal and stromal reactions [17]. Hyperhomocysteinemia also has a toxic effect on the endothelium of the hepatic vessels due to the production of significant amounts of free radicals and stress of the endoplasmic reticulum [13].

## Conclusions

Experimental hyperhomocysteinemia revealed changes at the ultrastructural level in all structural components of

the liver of young rats. Hepatocytes showed signs of moderate dystrophic changes. The nuclei were characterized by the presence of compacted chromatin, and the nuclear membrane had numerous intussusceptions. There were signs of pyknosis in some places. The mitochondria of liver cells contain numerous cristae with signs of destruction, their matrix is enlightened. The expansion of the tubules of the granular endoplasmic reticulum and tanks of the Golgi complex is characteristic. Accumulation of fat and glycogen in hepatocytes was revealed. The bile duct lumens and perisinusoidal spaces were dilated. The latter contained stellate macrophages, fat-accumulating cells, fibroblasts, collagen and elastic fibers. The data obtained by us by electron microscopic examination of the structure of the liver are compensatory-adaptive in nature and are inverse.

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progression of chronic liver diseases. *Ukrainian Medical Journal*, 3(101), 56-59.

#### **СУБМІКРОСКОПІЧНІ ЗМІНИ В ПЕЧІНЦІ ЩУРІВ МОЛОДОГО ВІКУ ПРИ ГІПЕРГОМОЦИСТЕІНЕМІЇ**

**Галаган Ю.В.**

На сьогоднішній день встановлено, що гіпергомоцистеїнемія відводиться значна роль у розвитку та прогресуванні багатьох захворювань. Накопичення гомоцистеїну виникає внаслідок порушення співвідношення між його продукцією та виведенням з організму. Печінка відіграє вагомое значення в обміні гомоцистеїну, оскільки в ній відбувається більша частина реакцій його трансметилування, а, отже, вона найпершою піддається негативному впливу. Метою дослідження є виявлення особливостей електронно-мікроскопічних змін структури печінки щурів молодого віку при гіпергомоцистеїнемії. Експериментальне дослідження проведене на 22 білих нелінійних молодих (1-2 місяці) щурах-самцях, яких було поділено на групу контролю та дослідну групу. Модель стійкої гіпергомоцистеїнемії створювали шляхом введення щурам дослідної групи тіолактону гомоцистеїну в дозі 200 мг/кг маси тіла інтрагастрально протягом 60 днів. Вивчення ультраструктурних змін в печінці щурів проводили за допомогою електронного мікроскопу ПЕМ-125К. Встановлено, що введення щурам тіолактону гомоцистеїну в дозі 200 мг/кг призвело до розвитку дистрофічних змін гепатоцитів. Зміни структури клітин печінки проявлялись у вигляді набряку цитоплазми та мітохондрій, деструкції мітохондріальних крист, розширенням канальців гранулярної ендоплазматичної сітки та цистерн комплексу Гольджі. Характерним є підвищення активності жиронакопичувальних клітин печінки та зірчастих макрофагів. У просвітах синусоїдних капілярів виявляли складні формених елементів крові, цитоплазма ендотеліоцитів мала ознаки набряку. Таким чином, при експериментальній гіпергомоцистеїнемії встановлені зміни на ультраструктурному рівні в усіх структурних компонентах печінки молодих щурів. Встановлені зміни мають компенсаторно-адаптаційний характер та є зворотними.

**Ключові слова:** гіпергомоцистеїнемія, гепатоцити, мітохондрії, макрофаги, синусоїдні капіляри.

#### **СУБМІКРОСКОПИЧЕСКИЕ ИЗМЕНЕНИЯ В ПЕЧЕНИ КРЫС МОЛОДОГО ВОЗРАСТА ПРИ ГИПЕРГОМОЦИСТЕИНЕМИИ**

**Галаган Ю.В.**

На сегодняшний день установлено, что гипергомоцистеинемии отводится значительная роль в развитии и прогрессировании многих заболеваний. Накопление гомоцистеина возникает вследствие нарушения соотношения между его продукцией и выведением из организма. Печень играет большое значение в обмене гомоцистеина, поскольку в ней происходит большая часть реакций его трансметилирования, а, следовательно, она первой подвергается негативному воздействию. Целью исследования является выявление особенностей электронно-микроскопических изменений структуры печени крыс молодого возраста при гипергомоцистеинемии. Экспериментальное исследование проведено на 22 белых нелинейных молодых (1-2 месяца) крысах-самцах, которые были разделены на группу контроля и исследовательскую группу. Модель стойкой гипергомоцистеинемии создавали путем введения крысам исследовательской группы тиолактону гомоцистеина в дозе 200 мг/кг массы тела интрагастрально в течении 60 дней. Изучение ультраструктурных изменений в печени крыс проводили с помощью электронного микроскопа ПЭМ-125К. Установлено, что введение крысам тиолактона гомоцистеина в дозе 200 мг/кг привело к развитию дистрофических изменений гепатоцитов. Изменения структуры клеток печени проявлялись в виде отека цитоплазмы и митохондрий, деструкции митохондриальных крист, расширением канальцев гранулярной эндоплазматической сети и цистерн комплекса Гольджи. Характерно повышение активности жиронакопительных клеток печени и звездчатых макрофагов. В просветах синусоидальных капилляров выявляли сложные форменные элементы крови, цитоплазма эндотелиоцитов имела признаки отека. Таким образом, при экспериментальной гипергомоцистеинемии установлены изменения на ультраструктурном уровне во всех структурных компонентах печени молодых крыс. Выявленные изменения имеют компенсаторно-адаптационный характер и являются обратимыми.

**Ключевые слова:** гипергомоцистеинемия, гепатоциты, митохондрии, макрофаги, синусоидальные капилляры.