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Dilorom B. Adilbekova  
*Tashkent Medical Academy, Tashkent, 100109, Uzbekistan*

Ravshan D. Usmanov  
*Tashkent Medical Academy, Tashkent, 100109, Uzbekistan*

Utkir M. Mirsharapov  
*Tashkent Medical Academy, Tashkent, 100109, Uzbekistan*

Dilorom A. Mansurova  
*Tashkent Medical Academy, Tashkent, 100109, Uzbekistan*

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MORPHOLOGICAL STATE OF EARLY POSTNATAL FORMATION OF THE ORGANS OF THE GASTROINTESTINAL TRACT AND LIVER IN OFFSPRING BORN AND RAISED BY MOTHERS WITH CHRONIC TOXIC HEPATITIS

Dilorom B. Adilbekova¹, Ravshan D. Usmanov², Utkir M. Mirsharapov³,
Dilorom A. Mansurova³, Hamidulla A. Rasulov⁴, Khabibulla H. Pulatov⁵

¹ MD, PhD, Department of Anatomy and Clinical Anatomy, Tashkent Medical Academy, Tashkent, Uzbekistan.
² MD, Professor, Head of the Department of Anatomy and Clinical Anatomy, Tashkent Medical Academy, Tashkent, Uzbekistan.
³ Senior Researcher, Professor, Department of Anatomy and Clinical Anatomy, Tashkent Medical Academy, Tashkent, Uzbekistan.
⁴ PhD, Department of Anatomy of the Tashkent Pediatric Medical Institute, Tashkent, Republic of Uzbekistan
⁵ Scientific researcher, Department of Anatomy of the Tashkent Pediatric Medical Institute, Tashkent, Republic of Uzbekistan

ABSTRACT

The processes of formation and development of the internal organs of mammals begin in utero, and end after birth. However, various pathological factors of the internal and external environment during pregnancy or lactation can adversely affect the processes of development of the structure and function of organs.

INTRODUCTION

The rapid development of the chemical industry, the improper disposal of chemical and nuclear waste, the widespread use of pesticides and other toxic chemicals not only caused negative environmental changes, but also caused acute and chronic toxic poisoning and damage to organs and tissues of humans and animals [1, 2].

It is known that the birth and upbringing of healthy children primarily depends on the state of the mother. This implies the relevance and importance of studying the influence of mother pathology on offspring.
The question of the effect of maternal liver pathology on pregnancy and on offspring has long attracted the attention of physicians, since it is often one of the causes of death of young children and often leads to a variety of severe injuries. [3, 4].

The question of the effect of maternal chronic toxic hepatitis on the morphological aspects of the early postnatal development and formation of the organs of the gastrointestinal tract of the offspring has not been sufficiently studied to date. [5]. Therefore, the goal of our work was to study the early postnatal development and formation of the organs of the gastrointestinal tract and liver in offspring born and raised by mothers with chronic liver pathology.

MATERIALS AND METHODS

The experiments were carried out on sexually mature female white outbred rats weighing 170-190 g. A model of heliotrin hepatitis in experimental animals was obtained by weekly administration of heliotrin with a calculation of 0.5 mg / 100 g of animal weight for 6 weeks. 10 days after the last injection, the males were planted in the males. The experimental animals were rat pups at the age of 3, 7, 14, 21.30 days of life, born and raised from female mothers with chronic toxic hepatitis. These study periods are consistent with the generally recognized division of the age periods in rats: neonatal period (1-5 days), lactation period (6-21 days). The work was carried out in accordance with the "Rules for the work using laboratory animals." Animals were decapitated under ether anesthesia. The control group consisted of rat pups born and nourished by healthy intact females, who, instead of heliotrin, were given the same amount of isotonic solution at the same time.

For the study, pieces were taken from the stomach, small intestine, colon and liver from control and experimental animals on the 3rd, 7th, and 21st days of postnatal life. The isolated material from the organs was fixed in a 10% solution of neutral formalin and Carnoy fluid. The resulting material was subjected to morphological. morphometric and electron microscopic research methods. To study intraorgan vessels, an intracardiac solution of black mascara was injected according to the original method of M.A. Kolesov through the left ventricle of the heart. Vascular enlightenment was carried out according to the method of A.G. Malygin. Pieces of sections after conducting them in twice saturated solutions of paraffin on xylene and pouring into paraffin with wax were installed on the pads. Serial sections 30–40 µm thick were made from them after pouring with a mass of Herot and 20–40 µm after pouring with ink. The diameter of the vascular lumen and the distribution density of microvessels were determined.

All obtained morphometric data were subjected to variational-static processing according to the Fisher-Student method in the modification of Ermolaeva-Biryukova using a software package on a computer. Differences satisfying P≤ 0.005 were considered significant.
RESULTS AND DISCUSSION

In the course of experimental studies, it was revealed the effect of chronic liver pathology in the mother on the postnatal morphogenesis of the organs of the gastrointestinal tract of the offspring. The experiments showed that toxic hepatitis of the mother adversely affects the postnatal growth, development and formation and the morphofunctional state of the vascular and tissue structures of the organs of the gastrointestinal tract and liver of the offspring, causing pathomorphological changes in their vascular and tissue structures, contribute to lag, delay in development processes and their becoming.

Studies have shown that the stomach in pups of the control group on the 3rd day of postnatal life has a thin, mucous membrane of light pink color with a grayish tint. The surface of the mucosa has a small number of low folds. All 4 membranes are distinguished in the wall of the stomach: the mucous membrane is the most powerful in thickness, the submucosa (loose, unformed connective tissue), the serous (mesothelium) and the muscle (thin, consists of smooth muscles) membrane. The surface of the mucous membrane and gastric fossa are covered with a single-layer cylindrical epithelium, the gastric fossa is less deep than in the control (intact) baby rats. The stroma of the interwell, fundus and pyloric glands contains a small amount of connective tissue cells. In the fundus glands, most of all are glandular cells proper, containing mucous secretory granules. These cells subsequently differentiate into additional cells. Edema and swelling, infiltration by mononuclear cells are detected in the mucous membrane.

Thus, the study of the postnatal formation of the wall layers of the fundus and pyloric stomachs of rat pups born and raised by mothers with CTG in the early suckler period allows us to establish that the processes of morphological development and formation of the stomach lag behind them than those in animals born and fed control by females. The thickness of all layers of the wall of the stomach is less than that of the control rat pups, which is due to the significantly shorter length of the gastric glands and the depth of the gastric fossa. In the stroma of the mucosa, lymphohistiocytic infiltration is noted.

After 2 weeks of life, the thickness of the mucous membrane of the wall of the stomach lags behind in the development of control animals - 1.2 times. The folded structure of the shell is clearly detected, on its surface - a moderate amount of mucus. In places, the folds of the mucosa are deformed, vacuolization and desquamation of the cells of the epithelial layer are determined on the thinned sections of the membrane. The gastric fossae are relatively narrow, lined with a prismatic, sometimes flat epithelium with a light cytoplasm and nuclei located in the basal part of the cells. In the glands, parietal, main and cervical cells in the fundus and supplementary cells in the pyloric stomach are clearly revealed. Parietal cells are concentrated mainly in the lower part of the necks of the glands. The inter-ferrous and especially inter-stromal stroma is infiltrated.
by mononuclear cells. The pit cells have a prismatic shape with elongated nuclei located at the same level. In places, the cells are swollen, the borders between them are erased. The number of parietal, main and parietal cells is less than in control animals. In the glands of the fundus and pyloric department there are more differentiated and poorly differentiated parietal and main cells. It should be emphasized that microorganisms appear in the lumen of the glands and on the surface of the mucosa that are in direct contact with the apical membrane of the main and parietal cells. Electron microscopically, the cytoplasm of the cells contains a complex of organelles. A distinctly large number of vesicles and mitochondria with less developed cristae. The endoplasmic reticulum and Golgi complex are well developed. A relatively large number of secretory granules containing electron-dense areas are detected.

In 21-day-old baby rats, the general architectonics of all layers of the wall of the stomach practically approach those of adult animals. Folds and pits are formed in the mucous membrane. But the morphometric indicators of all layers of the stomach are significantly behind the indicators of the control group. The depth of the fossae and the length of the glands of the mucosa are significantly less than in the control animals; the lumen of the gastric fossa is unevenly expanded. The interwell stroma is infiltrated by mononuclear cells. The number of parietal, main and parietal cells is less than the control numbers. In cervical and accessory cells, no significant changes were noted. Among the differentiated parietal and main cells, few and undifferentiated cells are found. The cytoplasm of the neck cells contains electron-dense vacuoles. In the lumen of the glands and on the apical part of the cells, many microorganisms are detected.

Thus, during breastfeeding, parietal main and cervical cells differentiate to perform specific secretory functions of the stomach. The lag of the thickness of all layers of the wall of the stomach from the index of control rat pups is due to the shorter length of the glands and the depth of the gastric fossa of the mucosa, and the thickness of the mucosa and serous-muscular membranes.

Intraorgan mucous membrane of the vessels in some places dilated and narrowed. The morphology and morphometric parameters of the microvessels of all the membranes of the wall of the stomach have changed significantly. The diameter of the capillaries is slightly different than in the control. Their wall is swollen and convoluted. Collector venules in places are somewhat enlarged and tortuous. The venule wall is still not clearly differentiated. Single reticular fibers and smooth muscle cells are revealed in it. The distribution density of the microvasculature of the mucous membrane is slightly less than in control animals. In some parts of the capillary network, the contours of the walls are fuzzy, there are microbleeds. Similar changes were detected in the microvessels of the submucosal and serous-muscular membranes.
By the 21st day of life in animals, pathomorphological changes persisted. The vessels of the mucous membrane and serous-muscular membranes are not uniformly filled with injection mass, the lumen diameter of the arterioles has slightly increased, the vessels are dilated, sinuous, their wall is swollen. The capillary diameter parameters are slightly larger than the control groups. Electron microscopically, their wall consists of 3 layers: the endothelial layer with the basement membrane, the pericyte layer and the outer layer of adventitious cells and thin reticular fibers. The distribution density of the vessels of the mucous membrane is 1.2-1.4 times less than in control animals. In capillaries, endothelial cells slightly swell into the lumen of the vessels, the lumen surface is uneven, the cytoplasm is light with a large number of intracellular organelles.

In 30-day-old baby rats, the general architectonics of all layers of the wall of the stomach practically corresponds to that in adult animals. Folds and pits are formed in the mucous membrane. But the morphometric indicators of all layers of the stomach are significantly behind the indicators of the control group. The depth of the fossae and the length of the glands of the mucosa are significantly less than in the control animals; the lumen of the gastric fossa is unevenly expanded. The interwell stroma is infiltrated by mononuclear cells. The number of parietal, main and parietal cells is less than the control numbers. In cervical and accessory cells, no significant changes were noted. Among the differentiated parietal and main cells, few and undifferentiated cells are found. The cytoplasm of the neck cells contains electron-dense vacuoles. In the lumen of the glands and on the apical part of the cells, many microorganisms are detected.

In 30-day-old baby rats, pathomorphological changes in the microvessels of the stomach wall gradually subside in places, and still persist in places. In the wall of arterioles, thickenings and swelling are detected in places. Elastic fibers and single smooth muscle cells are detected in the wall of venules and veins. The processes of cell differentiation are still ongoing. Among poorly differentiated cells, short elastic fibers are detected. Venous vessels of the wall of the stomach in places are microaneurysmically dilated, sinuous, filled with blood. The capillaries are somewhat narrowed in places, tortuous, there are microextravasases. Indicators of the density of distribution of blood vessels of the mucous membrane and serous-muscular membrane are less than indicators of control groups of animals.

The pathology of the mother’s liver negatively affected the state of the offspring not only during the neonatal period, but also in the process of postnatal development. First of all, 3-4 days later there were signs of the general development of animals: auricle detachment, the appearance of a coat and the realization of a standing pose. A significant increase in postnatal mortality was observed.
In rat pups born to mothers with chronic toxic hepatitis, in the early 3-7 days of postnatal development in the mucous membrane of the small intestine, some thinning was observed in comparison with control animals. The mucous membrane is represented by many folds and villi. Its cover consists of squamous epithelium, on the surface of which a moderate mucus content is determined. Moderately pronounced dystrophic changes in the villi were established: their tortuosity, abundant vacuolization of the cytoplasm of integumentary epithelial cells, and deformation of its nuclei. In the stroma of the villi, foci of lymphohistocytic infiltration are often found. The length of the villi and the depth of the crypts, the number of mitotically dividing cells is less than in the control. In the apical part of the cytoplasm of cells there is a large number of secretory granules. The vacuolated Golgi complex, expanded profiles of the granular endoplasmic reticulum, a large number of mitochondria and vesicles are revealed. The submucous membrane is loosened in places due to edema and lymphohistiocytic infiltration. Foci of micronecrosis of connective tissue are found. The vessels of this layer are sometimes filled with blood and with perivascular hemorrhages. The muscle layer consists of two layers: the inner one is circular, the outer one is longitudinal. It is swollen, swollen and loosened, has an uneven thickness. Vacuolated muscle cells are defined in places. Ultrastructural studies indicate a tendency to some lag, slowing down the processes of differentiation of parietal and main gland cells. Electron microscopy in the apical part of the cytoplasm of surface-dimple cells contains a large number of secretory granules, a vacuolated Golgi complex, and expanded profiles of the granular endoplasmic network of mucocytes. In parietal cells, a high content of mitochondria with a dense and increased content of vesicles; intracellular secretory tubules, closed in the cytoplasm, delayed secretion in parietal cells. Thus, ultrastructural studies indicate a tendency in a certain lag, a slowdown in the processes of differentiation of parietal and main gland cells. The intraorgan vessels of the mucous membrane are narrowed in some places and dilated in some places. The architectonics of microvessels of all layers and sections of the stomach without significant changes. The vascular wall is swollen and sinuous in places. Marked expansion, tortuosity and blood filling of the collector venules, the walls of individual vessels are edematous, with erased contours (Fig. 1)
In the organ vessels of the small intestine, dilated, sinuous, full-blooded venous vessels with microstasis were detected in some places. The submucous membrane is loosened in places due to edema and lymphohistiocytic infiltration. Foci of micronecrosis of connective tissue are found. The vessels of this layer are sometimes filled with blood and with perivascular hemorrhages.

On the 14th day of the postnatal development of animals, the described pathomorphological changes persisted in all layers of the intestine. The delay in morphological development is expressed in smaller sizes of villi and crypts than in the control, and a decrease in the mitotic activity of intestinal epithelial cells. The mucous membrane is thinned in places, that is, of uneven thickness. In thinned areas of the mucous membrane, vacuolization, necrosis and desquamation of the surface epithelium are determined. Locally sinuous villi, stick together. The formation of the muscle plate of the intestinal mucosa lags behind the control dates. A large number of differentiated and undifferentiated cells are detected. Electron microscopically, their cytoplasm contains a complex of organelles, a large number of vesicles and mitochondria. In the submucosa, edema, infiltration by mononuclear cells is preserved. In the muscle membrane, some looseness of the intermuscular space and an increase in the foci of cambial cells are noted.

On the 21st day of observation, there was a tendency to some subsidence of pathomorphological changes, they gradually leveled, however, in some animals the involution of such deviations did not subside, moreover, it intensified, especially in the proximal sections of the small intestine. In some animals, the villi in the mucous membrane are sometimes deformed, fused together by the tops or sides. The stroma of some villi is edematous and infiltrated by mononuclear cells. Morphometric indicators of the thickness of the mucous membrane, the depth of the crypts, the height of the villi did not reach the indices of the control animals. The number
of mitotically dividing cells was also less than in the control and than the previous dates. The submucosa is edematous and infiltrated, the lymphoid follicles are hypertrophied. To a lesser extent, the Brunner glands changed; only in places, in areas of edema and infiltration, moderate destructive changes (expansion of the gland of the glands, flattening of the secretory epithelium) were detected. The muscle layer of uneven thickness, loosened, nerve bundles in places hypertrophied.

In the submucosa, the blood vessels are dilated and convoluted. In the mucous membrane, a slight thinning of endotheliocytes and a few pinocytotic vesicles, often located in separate groups. The basement membrane is swollen, loose, sometimes absent. A thickened, loosened basement membrane is revealed in the wall of venules (Fig. 2).

![Fig. 2. Blood capillary of the mucous membrane of the jejunum of 21-day-old rat pups. The basement membrane is loosened, endotheliocytes are thinned. TEM. SW 10500](-figure2.jpg)

In the lumen of blood vessels in places there are aggregated red blood cells and platelets (Fig. 3).

![Fig. 3. Blood vessels of the submucosa of the duodenum in 21-day old rat pups. The basement membrane is loosened, in the lumen of the vessel is the aggregation of blood cells. TEM. SW 10500 ×](figure3.jpg)

In 30-day-old pups, signs of impaired general development were significant fluctuations in the severity of the pathomorphological changes in the intraorgan vessels of the GMSC and the nature of their age dynamics. In some young rats, the morphometric indices of the vessels
approached the control indices, as a result of which the organ microstructure also returned to normal. But in most animals, pathomorphological changes still persisted, and in some they progressed, especially in the proximal intestinal wall. The wall of arterioles is thickened in places. In the intima of the vessels, “pillow-shaped” bulges are revealed. The inner elastic membrane is somewhat thickened, exfoliates in places of pillow-like bulges. A well-developed layer of smooth muscle cells is detected. The diameter of the lumen of the capillaries and the density of the vessels of the mucous membrane differ from the control indicator. In places, the capillaries of the mucous membrane are spasmodic, and the vascular zones are visible. Similar processes are detected in the vessels of the submucosa and serous-muscular membrane (Fig. 4). Venous vessels are somewhat dilated, the diameter exceeds the control. In some places, accumulation of mast cells is detected around the vessels.

![Fig. 4. Blood vessels of the serous-muscular membrane of the small intestine in 30-day-old rat pups. The vessels are dilated, tortuous. Pouring vessels with a mass of Herot. Object 20. eyepiece. 10.](image)

Thus, a study of the postnatal development and formation of the small intestine of offspring born and nursed by mothers with CTG showed that damage to the mother’s liver violates the morphofunctional state of the gastrointestinal tract, causes destructive changes in them: the growth rates of the crypt-villus system components lagged, the numbers of mitotically dividing cells decreased, and the proliferation and differentiation of cells slowed down. These changes subsequently led to a delay and lag in the growth and formation of the vascular tissue structures of the stomach and intestines. Although these disorders in the postnatal period of development are gradually compensated, however, in most animals they were persistent and were observed until the end of the study period.

Our studies showed that in the early stages of development (3-7th day after birth), rat pups morphologically colon wall is expressed quite well. The mucous membrane in the proximal regions is represented by pronounced villus-like folds of various shapes and sizes. The integumentary epithelium in all departments consists of flat cells, the nuclei of which are located...
in the basal part of the cells. The borders between the cells are fuzzy, the cytoplasm of the cells is wide, bright. The crypts are shallow, their stroma is edematous, infiltrated by mononuclear cells.

After 7 days, the indicators of the depth of the crypts in the proximal, middle and distal parts of the intestine were slightly less than the control numbers. In the upper sections of the crypts, the limbic cells have swollen in places, their borders are obliterated. In individual cells, nuclear deformation and vacuolization of the cytoplasm are observed. Enlightened crypts expanded. Stroma of crypts loosened, infiltrated by mononuclear cells. The number of mitotically dividing cells is less than in control animals. Electron microscopic cytoplasm of the cells is light, contains a complex of organelles. Swollen mitochondria with a light matrix are found. The vacuolated Golgi complex is detected, and lymphocytes between the epithelial cells. The basal membrane is of moderate density, somewhat thinned. The number of goblet cells exceeds the control numbers. They are at various stages of secretion. Indicators of the distribution density of microvessels in 3-7-day-old rat pups are slightly less than in control animals. The wall of venules in places varicose dilated, tortuous. The diameter of the lumen of the venous vessels is greater than the control. Among their poorly differentiated cells, short elastic fibers and single smooth muscle cells are determined in their wall. Mucopolysaccharides and mast cells are concentrated around the vessels. Due to the close morphofunctional and neurohumoral relationship of the digestive organs with each other, pathological changes in one organ cause disturbances in the other.

After 14 days of postnatal development of rat pups, indicators of the thickness of the mucous and serous-muscular membranes of the colon, the depth of the crypts and the number of cells in the crypts were less than in the control animals. The integumentary epithelium is flattened, in places with pycnotically modified nuclei. Detachment of the epithelial layer was encountered. Cells are polymorphic, swollen in places, with a light cytoplasm. Separate crypts in the middle and lower third are enlarged; their stroma is infiltrated by lymphohistiocytic cells (Fig. 5).

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Significant violations were found in the microstructure of the liver in newborn rat pups (3-7 days). Hepatocytes were loosely and randomly, separated by wide and full-blooded sinusoidal hemocapillaries. In many hepatocytes, hydrolytic dystrophy was observed. In individual cells, pycnosis and nuclear lysis were observed.

It should be noted that all the above morphological changes in the vascular and tissue structures of the small and large intestines were focal in nature.

Our studies once again prove that due to the close interconnection of the functional system “mother-fetus”, the fetus in the mother’s body is an additional, new organ. Because of this, any pathology of the mother before and during pregnancy leads to pathology of the offspring organs not only in the prenatal, but also in the postnatal period of development.

Thus, the data of our studies showed that the pathology of the liver in the mother adversely affects postnatal growth, development and formation, and the morphofunctional state of the organs of the gastrointestinal tract and liver of the offspring. Analyzing these processes, we believe that a violation of the detoxification function of the mother’s liver is of great importance here. The fetal liver is not yet ready for sufficient detoxification of metabolic products. It is clear that, in this case, accumulation in tissues, including the liver, of substances (pyruvic acid, lactic acid. Products of free radical oxidation and metabolites, etc.), which has a cytotoxic effect, is possible. A harmful effect on the internal organs of the developing fetus can be exerted by such products that, when the permeability of the placenta is impaired, penetrate the fetus, and normally undergo detoxification in the mother’s liver and do not enter the fetus. [6, 9, 10, 11]. The consequences of this effect are noted already in the early postnatal period of development in the form of a gradual dystrophic process in the vascular-tissue structures of the studied organs. Another reason for the pathomorphological changes in the offspring is apparently due to certain immunopathological changes in the body, because the protein products of decaying hepatocytes cause an autoallergic reaction, destructive changes in the internal organs indicate the possibility of such a mechanism. In addition, the structural disturbances of the digestive system organs and their microvasculature established by us in rat pups born to mothers with ChTG may be due to a deficiency of plastic and a number of biologically active substances for the fetus during the embryonic period of development. This deficiency occurs when there is a violation of the function of the mother’s liver, placental insufficiency, under the action of products of impaired metabolism. In the blood of the mother as a result of inhibition of the antitoxic function of the liver, distorted metabolic products accumulate that affect the embryos in the prenatal period of development. On the other hand, developing hepatic cell failure leads to changes in the mother's body - this is reflected in a change in the quantitative and qualitative composition of mother’s breast milk and subsequently these factors affect the
processes of postnatal growth, development and formation of the offspring organs. The combination of the above factors, in our opinion, determines the violation of histogenesis and morphogenesis in the fetus, in postnatal ontogenesis, gradually developing dystrophic processes, slowing and lagging of the processes of growth, development and formation of organs and systems. Considering that the animals were replanted on the 10th day after the drug was administered to females (hepatotoxin is absent in the blood of females during this period), we believe that the above pathomorphological changes in the vascular-tissue structures of the stomach, small and large intestine, and liver are apparently the cause. First of all, there is a shortage of plastic, trophic and energetic material in the fetus during the prenatal period of development, due to the pathology of the mother’s liver, and not the direct effect of hepatotropic poison on the fetus. At the same time, other factors are not excluded, such as impaired antitoxic function of the liver and the resulting metabolic products and their metabolites in the mother’s blood entering the fetus through the placenta, changes in the quantitative and qualitative composition of mother’s breast milk. All this necessitates the development of evidence-based therapeutic and preventive measures in order to prevent pathology in children born and raised by mothers with liver pathology.

CONCLUSION

1. Chronic toxic damage to the mother’s liver negatively affects the processes of postnatal development and the formation of tissue structures of the organs of the gastrointestinal tract of the offspring.

2. Hepatotoxins introduced into the mother’s body before pregnancy and formed during hepatitis, getting into the bloodstream and then into mother’s milk, contribute to the development of inflammatory-reactive changes in the vascular tissue structures of the gastrointestinal tract in the body of the offspring in early life postnatal development.

3. Pathomorphological changes in the vascular and tissue structures of the gastrointestinal tract of offspring subsequently lead to a delay in the processes of postnatal formation and development of organs and systems as a whole.

4. All this necessitates the development of evidence-based therapeutic and preventive measures in order to prevent pathology in children born and raised by mothers with liver pathology.