PECULIARITIES OF PRENATAL INFLUENCE OF A NUTRITIONAL FACTOR ON THE STRUCTURAL AND FUNCTIONAL STATE OF THE LIVER OF NEWBORN RATS

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Summary

Disease of the digestive system occupies one of the first places in the structure of morbidity and mortality in the population of Ukraine and EU countries. The alimentary factor (diet with an excess or deficiency of nutrients) is of leading importance Among the factors that cause liver damage in the mother-fetus system.

The purpose of this study was to establish the effect of excess or insufficient content of nutrients in the mother's diet on the structural and functional state of the liver of newborn rats.

Materials and Methods. To achieve this goal, 20 female rats of the WAG population were used; they were divided into 3 groups: group 1 (control) - rats were in standard vivarium conditions and received a basic diet; animals of the 2nd group received a diet with excess nutrients; rats of the 3rd group received a nutrient deficient diet. The offspring of rats were hatched from experiment through decapitation straightaway after birth. Was a complex of morphological and biochemical studies of liver tissue was carried out.

Results. When analysing micro preparations of liver tissue, a similarity in the nature of the induced changes in the organ rats of the 2nd and 3rd groups in the form of moderately pronounced discomplexation of beamed-radiary structure and expansion sinusoids were revealed. The difference was that in rats of the 2nd group, mainly around the zone of portal tracts, small extramedullary hematopoiesis foci; and in rats of the 3rd group, multiple small extramedullary hematopoiesis foci that indicated more pronounced hypoxia were determined. Thus, it can be noted that the greatest damage to the fetus liver was caused by nutritional deficiency in the mother's diet.

When studying the fractional composition of lipids in liver homogenates of newborn rats, the following dynamics of changes was discovered: in animals of the 2nd group - an increase of cholesterol and triglycerides levels, with a decrease of PL level, and in rats of the 3rd group, a decrease in almost all fractions of lipids cholesterol, triglycerides, and NEF A (non-esterified fatty acid). The obtained data suggested that, most likely, such dynamics of changes in lipid metabolism parameters is associated with the inclusion of epigenetic programming mechanisms in the mother-fetus complex.

Conclusions. Therefore, based on conducted research, we can do conclusion about the negative impact of the alimentary factor (nutrient deficiency) on the structural and functional state of the liver of newborn offspring of rats.

Keywords: liver, newborn rats, diet with an excess of nutrients, alimentary deficiency of nutrients, structural and functional state of the liver, fractional composition of lipids.

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1. Introduction

Every year in the EU countries and Ukraine, an increase in the prevalence of diseases of the digestive system, in particular, the liver, is recorded. Special attention of doctors and scientists is caused by the increase in the level of maternal and infant mortality. One of the factors leading to this is extragenital pathology (especially, liver diseases). Thus, according to the literature, it is known that the maternal mortality rate due to aggravated pregnancy in Ukraine exceeded the European values by 1.3-1.6 times.

Non-alcoholic fatty liver disease (NAFLD) plays a leading role in the prevalence of liver diseases. According to statistics, NAFLD is diagnosed in 20-40% of the population of European countries. In the United States, the prevalence of NAFLD was found to be in 32% of patients *(Plokhotnichenko O.A., 2018)*, while population-based studies in Japan, China, and Italy showed that the prevalence of NAFLD ranged from 13 to 25% *(Plokhotnichenko O.A., 2018: 8)*. In children and adolescents in the United States, this figure was 10%, and in Ukraine at the moment there are no reliable statistical data on the prevalence of NAFLD in these age groups *(Dikan I.M., 2021)*.

A significant role is occupied by the alimentary factor among the factors that can negatively affect the condition of hepatobiliary system, mother and fetus.

So, many scientific studies have proven unfavourable influence of hypercaloric diet nutrition on the state of the gastrointestinal tract organs of adult male and female rats, which was manifested by disorders of fat metabolism and distinct morphological changes in the organs of their digestive system *(Buettner R., 2006; Kobayasi R., 2010)*. The data obtained from the analysis of literary sources indicate that the complications that develop in rats that consume excessive amounts of fat are manifested by hypertrophy and fibrosis of the heart, myocardial necrosis, and liver steatosis *(Buettner R., 2006; Kobayasi R., 2010; Rutledge A.C., 2007; Chang K.C., 2007)*. In mice, feeding a high-fat diet also induces an increase in systolic blood pressure and the development of endothelium dysfunction *(Shapiro A., 2008)*.

A diet high in animal or vegetable fat has been shown to lead to the development of obesity, hyperinsulinemia and hyperglycemia, as well as impaired glucose tolerance, increased levels of cholesterol, and low density lipoproteins *(Wentzel P., 2019)*.

In addition, it has been established that the consumption of an excess amount of carbohydrates (fructose) induces the expansion of the ventricles of the heart, hypertrophy and a decrease in their contractile function, as well as liver steatosis. *(Stothard K.J., 2009; Blomberg M.I., 2010; Nakagawa T., 2005)*. In rats, fructose ingestion causes damage to the renal tubules, deposition collagen in the interstitium, and increased macrophage infiltration together with proliferation and hyperplasia of the renal proximal tubules, and also contributes to leptin resistance without change in weight body and obesity *(Shapiro A., 2008; Blomberg M.I., 2010)*. It should also be noted that there was an increase in the concentration of urinary acids and triglycerides in blood plasma without significant changes in the concentration of cholesterol *(Nakagawa T., 2005)*. It is proved that rats of Wistar line who received commercial feed for 6 weeks together with standard diet, used 10% fructose solution as drinking water, showed development key signs characteristic of metabolic syndrome that is hyperglycemia and visceral obesity. In addition, they revealed a trend towards an increase in body weight, an increase in the concentration of total cholesterol and triglycerides in blood serum *(Nakagawa T., 2005)*.

The analysis of literary sources also revealed the negative impact of nutrient deficiency, which was determined by activation of lipid peroxide oxidation processes (LPO) on the cells of the digestive system *(Campisano S., 2019; Campisano S.E., 2017)*.

Thus, foreign researchers have proven negative influence of proteins of scarce diet on the morphological and physiological features of the functioning of the liver *(Campisano S., 2019)*. For example, after 60 days reception of proteins of scarce diet in the offspring of rats (males), a decrease in body weight and liver mass, in addition, an increase in activity of serum glutaminepyruvic transaminase, as well as the cholesterol and triglycerides content in the liver were found *(Campisano S.E., 2017)*. Additionally, researchers discovered liver dysfunction, which was characterized by promoted expression of γ-glutamyl transferase *(Du J. E., 2020)*.

Thus, it can be noted that an active study of the influence of the alimentary factor on the state of the gastrointestinal tract is currently being carried out, but its role in liver damage in the mother-fetus system remains insufficiently covered.

The aim of our study was to determine the effect of excess and insufficient amounts of nutrients in the mother's diet on the structural and functional state of the liver of newborn rats.

2. Materials and methods

The study was performed on 20 female rats of the WAG population, which were divided into three groups: group 1 was a control, consisted of rats that received a basic diet before the replanting of males and during pregnancy and were under standard vivarium conditions.

Animals of the 2nd group received a diet with an excess of nutrients, which consisted of 3g of proteins, 2.0g of fats and 25g of carbohydrates, with an energy value of -130 kcal, in a total amount of 30g per rat per day.

Rats of the 3rd group received a nutritionally deficient diet, which consisted of 1.02 g of proteins, 0.64 g of fats and 8 g of carbohydrates, with an energy value of 41.84 kcal, in a total amount of 9.66 g per rat per day.

Research was performed in compliance with the rules and international recommendations of the European Convention for the Protection of Vertebrate Animals Used for Experiments or in other scientific purposes *(Strasbourg, 1986)*. The offspring of rats were withdrawn from the experiment immediately after birth through decapitation.

Morphological examination of the liver tissue was carried out in accordance with generally accepted methods *(Avtandilov G.G., 1990)*. Fractional composition of lipids in homogenates of liver fabrics was determined using spectrophotometric method: cholesterol (CS), phospholipids (PL), triglycerides (TG) and NEF A (non -esterified fatty acid) using a set of reagents from Olveks (Russia).

Statistical analysis of received data were collected using theprogram Graph 5 PadPrism. To determine the significance of differences, the Man-Whitney U criterion was used.

3. Results

Visually, the liver of the 2nd and 3rd groups did not differ from the control one.

A comparative analysis of liver tissue micropreparations, morphological changes in the organ was revealed, which were most pronounced in newborn rats of the 3rd group. So in rats of the 2nd and 3rd groups, moderate discomplexation in the beamed-radiary structures of

liver was microscopically discovered; sinusoids have been expanded. In rats of the 2nd group, small extramedullary hematopoiesis foci, mainly around the portal tracts zone, were discovered (Fig. 1), and in rats of the 3rd group, there are multiple small extramedullary hematopoiesis foci (Fig. 2).

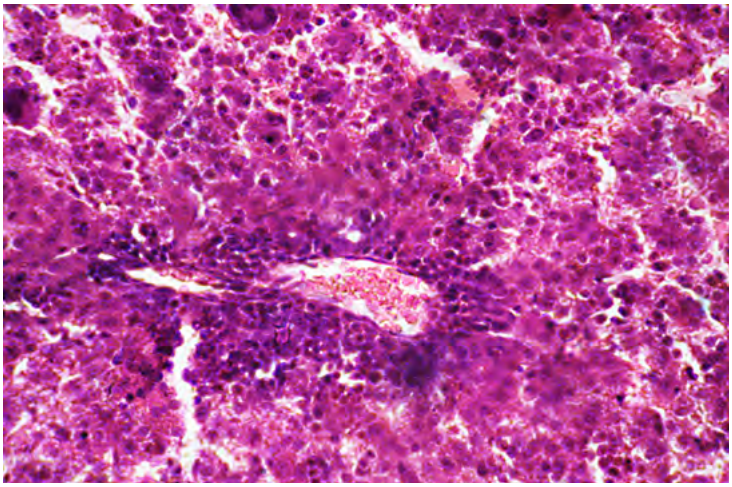


Fig. 1. Micrograph of the liver of a newborn 2nd group rat
(Staining with hematoxylin and eosin × 200)
Hepatocytes with granular cytoplasm and nuclear hyperchromia. Extramedullary hematopoiesis foci around portal tracts

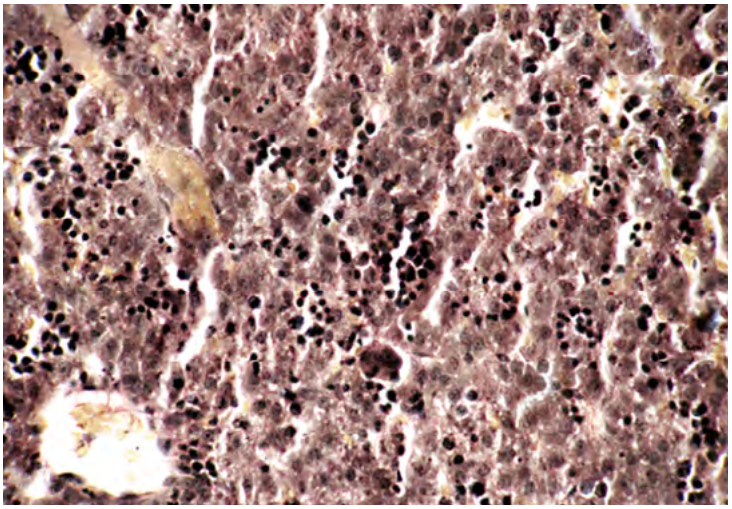


Fig. 2. Micrograph of the liver of a 3rd group newborn rat.
(Staining according to the van Gieson method × 200)
Pyknotic hepatocytes with granular cytoplasm and nuclear hyperchromia.
Multiple foci of extramedullary blood formation

The data obtained indicate that the rats of the 3rd group were in conditions of more pronounced hypoxia. In both groups, dual-core hepatocytes were determined for the entire field of view; this indicated a slight activation of the regenerative activity of the organ.

The stroma of the portal tracts in rats of the 2nd group (Fig. 3) had a normal appearance and was represented by thin, loose oriented collagen fibers. The data obtained suggest that due to excessive dietary intake during pregnancy, the liver of mother rats suffered more than that of newborn rats, thus having protective value in relation to defeat offspring liver.

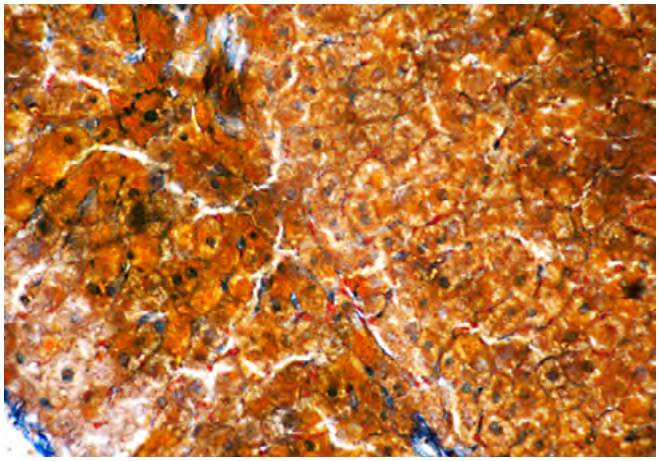


Fig. 3. Micrograph of the liver of a 2nd group newborn rat.
(Staining by the method of Mallory × 400)
Moderate proliferation of the stroma of portal tracts

In rats of the 3rd group (Fig. 4), the stroma of the portal tracts was moderately proliferated and presented by loosely oriented collagen fibres, which indicated a significant degree of organ damage in rats that were under conditions of prenatal nutritional deficiency.

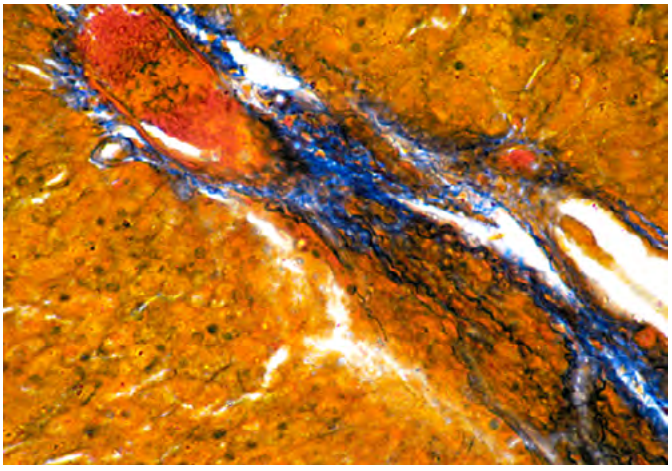
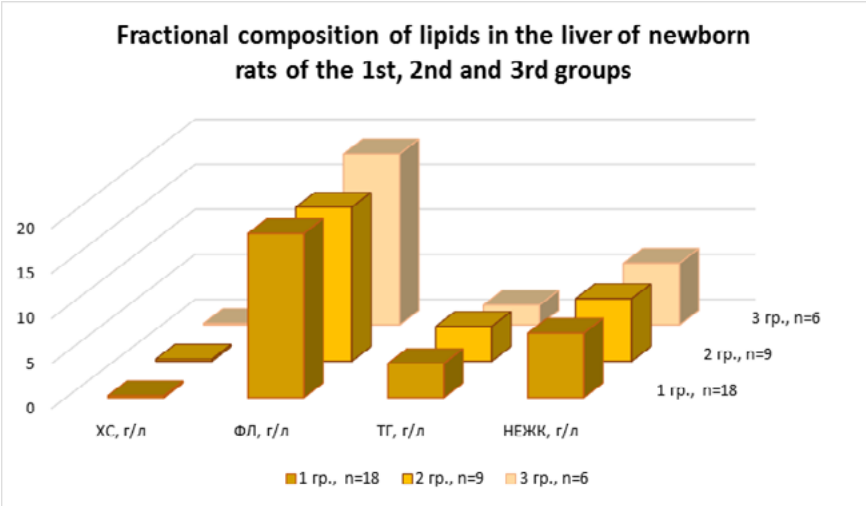


Fig. 4. Micrograph of the liver of a newborn 3rd group rat.
(Staining by the method of Mallory × 400)
Proliferation of the stroma of the portal tracts



гр	Group
ХС	Cholesterol
г/л	g/l
ФЛ	Phospholipids
ТК	Triglycerides
НЕЖК	NEF A (non-esterified fatty acid)

Fig. 5. Study of the fractional composition level for lipids in homogenates of newborn rat liver of control, and 2nd and 3rd groups

When studying the fractional composition of lipids in liver homogenates of the offspring of the 2nd group rats, a trend of change in total cholesterol, phospholipids, and triglycerides indicators was revealed: if the content of cholesterol is increased by 9.6% ($p < 0.05$), the content of phospholipids is reduced by 6% ($p < 0.001$), and the content of triglycerides is increased by 2.1% ($p > 0.05$) relative to NEF A (non -esterified fatty acid) level, then in newborn rats they are lowered. In rats of the 3rd group, the opposite dynamics of shifts was observed, which consisted in a decrease of cholesterol level by 21.01%, a decrease of triglycerides levels by 41.69% and a decrease of NEF A level by 4.47%. The data obtained indicate that the most negative impact on the structural and functional state of the liver in the mother-fetus system a diet with a deficiency of nutrients and methionine and choline had.

An important feature of the negative influence of the alimentary factor is that one of the mechanisms for the occurrence of damage in the mother-fetus system is intrauterine programming, which is realized through changes in the functioning of some organs and systems. In this case, this leads to the development of significant anomalies in the function of the liver of rats (especially those rats that were under conditions of intrauterine nutritional deficiency), which is primarily associated with changes in the expression of genes responsible for regulating the activity of enzymes that characterize liver function *(Kovtun O.P., 2014)*.

Therefore, if to generalize the data concerning to similarities of pathogenetic chain of liver damage due to the negative impact of alimentary factors, then it is necessary to note that

it is characterized by stereotypical events because any factor, which action requires occurrence of adaptive reactions, can be considered as the stressor that triggers a cascade of stereotypical reactions in the body *(Mathias P.C., 2014)*.

4. Conclusions

Therefore, based on the data obtained, it can be concluded that the greatest damage to the liver was caused by prenatal nutritional deficiency accompanied with moderately pronounced changes in the organ and functional abnormalities. In addition, the identified changes suggest the inclusion of epigenetic mechanisms, which in the future can lead to the formation of a variety of functional and organic pathology of a hepatobiliary system as the offspring grow.

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