Effect of amino acid imbalance in the nutrition on the morphology of animal liver

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Abstract. The article considers the influence of various forms of amino acid imbalance in diets on morphological changes in the liver of laboratory animals. An imbalance of amino acids caused a number of similar changes in the liver of animals. Liver sections revealed that hepatocytes had smoothed borders and areas with signs of granular dystrophy. The sinusoids and inter-protein spaces were overflowed with blood moulds. Small groups of lymphohistiocytic cells were presented in the parenchyma. Violation of the liver microcirculation contributed to the violation of metabolic processes in hepatocytes. Most of the hepatocytes were filled with yellow-orange fat droplets and had atrophied cytoplasm with nuclei shifted to the periphery. There were single cells with glycogen granules as well. The content of nucleic acids in tissues is an important indicator of the intensity of protein synthesis. Thus, in liver sections of groups 1, 2, 3 and 4, the cytoplasm of hepatocytes was less colored than in the control group, which indirectly indicates an increase in the intensity of protein synthesis in liver cells. Hemodynamic disorders in the form of dilated blood vessels and the appearance of stasis in groups 1, 2, 3 and 4 lead to difficulties in the penetration of amino acids to the sites of protein synthesis and, as a result, to a violation of metabolic processes in parenchymal liver cells.

1. Introduction
A direct relationship was found between the balance of amino acids in the diet of animals and metabolic disorders, in particular, excessive fat deposition in the liver due to reduced lipid transport to the blood [1]. Lack of lysine in the diet causes not only fat infiltration of the liver, but also negatively affects the condition of the bone marrow, disorders of hematopoiesis [2].

Excessive fat deposition in the liver of animals is also caused by a lack or excess of methionine in the diet [3, 4], a deficiency of threonine and tryptophan [5, 6].

2. Method
The aim of our research was to study the comparative effect of the imbalance of various amino acids in the diet of laboratory rats on liver morphology.

Experiments were performed on the white Wistar weaning rats. 6 groups of 14 animals (7 males and 7 females) were formed in each group. The animals were kept in the same conditions during the experimental period which lasted 14 days.
The first experimental group received a basic mono-grain diet that was not balanced in amino acids (50% of barley, 35% of wheat, 15% of corn), and 2, 3, 4 and 5 - an unbalanced diet. A mixture of essential amino acids was added to the norm of needs to the main diet, at that the diet of the second group lacked lysine, the third – methionine, the fourth – threonine, and the fifth – tryptophan. Animals of the sixth (control group) received all essential amino acids. The diets of all groups included 2% vitamin mixture, 3% mineral mixture and 2% sunflower oil.

14 days after the start of the experiment, the rats were decapitated. Liver samples for morphological studies were fixed in a 10% solution of formalin and Carnois liquid. Then they were washed with water and sectioned on a freezing microtome. For the total detection of lipids, sections were stained with sudan III. Sections with a thickness of 5-7 microns were prepared from paraffin blocks on a sledge microtome, which were stained with hematoxylin-eosin. Glycogen was detected by the PAS reaction with amylase control, the Einarson RNA method, and the Felgen DNA reaction [7, 8]. All preparations were examined under the BRM-3 microscope with an eyepiece magnification of 7, and the lens magnification of 10, 20, and 40.

3. Results and discussion

An imbalance of amino acids caused a number of similar changes in the rat liver. The most significant changes were found in the liver of rats kept on an unbalanced diet of amino acids, with an imbalance of lysine, methionine and threonine. The tryptophan imbalance led to changes that were not permanent. Thus, the rats of groups 1, 2, 3 and 4 had pale, flabby livers. It was revealed that the hepatocytes had smoothed borders and areas with signs of granular dystrophy when examining sections of the liver stained with hematoxylin-eosin. The sinusoids and interstitial spaces were filled with shaped blood moulds. Small groups of lymphohistiocytic cells were present in the parenchyma. Violation of microcirculation in the liver led to a violation of metabolic processes in hepatocytes. Most of the periportal hepatocytes were filled with drops of yellow-orange colored fat and had, as a rule, atrophied cytoplasm with nuclei displaced to the periphery. There were single cells with glycogen granules as well.

It is known that the content of nucleic acids in the tissues of internal organs is an important functional indicator, in particular protein synthesis. A comparative study of preparations obtained from the liver of experimental and control rats groups showed that the amino acid imbalance affects the intensity of the sections color during the Einarson reaction. Thus, the cytoplasm of hepatocytes stained with galloxylin-chromium alum in the liver sections of groups 1, 2, 3 and 4, was weaker than in the 6 (control) group.

No significant difference was found in the intensity of nucleus staining. This indicates a reduced content of nucleic acids in the cytoplasm in comparison with the nucleus.

The changes were less pronounced and manifested as focal granular dystrophy of hepatocytes in the rat’s liver of group 5. There was a tendency to accumulate fat in the form of small drops in the cytoplasm of individual liver cells. The level of glycogen in the liver was slightly reduced in comparison with the control. The PAS reaction revealed the glycogen diffusely in the form of a powdery grain or small granules. No significant and permanent differences were found when the rats liver sections of group 5 were stained for the content of nucleic acids.

The exception was the rats that received a balanced amino acid diet for 14 days. Their livers were dark cherry-colored and regular in shape, size and consistency. Hepatocytes had an oxyophilic color and a normal ratio between the nucleus and cytoplasm when sections were stained with hematoxylin-eosin. The cores are of moderate size with a clear border. Fat infiltration of hepatocytes was not detected in sudan III staining. The PAS reaction revealed that most of the hepatocytes were filled with glycogen clumps. Staining for nucleic acids revealed their main localization around the nucleus and along the cell periphery.

The fluctuations in its intensity were less pronounced and unstable when other structures of the liver tissue were stained with galloxylin-chromium alum.
By the Felgen reaction, the DNA was colored red-purple. The most intense and uniform color was found in the nuclei of connective tissue cells, Kupffer cells, and blood leukocytes. The nuclei of hepatocytes and epithelial cells of the bile ducts were stained weaker and less evenly. The content of DNA in the liver cells of the experimental and control groups was without any natural differences.

The results of morphological studies of the rat liver were compared with the indicators of their growth and development. The growth of animals on unbalanced diets (groups 1, 2, 4) was accompanied by a significant decrease in the average daily growth.

In groups 1, 2, and 4, the average daily gain was 1.37 g, 1.9 g, and 1.16 g, respectively, with 3.84 g in the control group. This indicator was close to the control in group 3, (3.65 g vs. 3.84 g), and in the fifth group it even exceeded it (4.1 g). This may have been due to better feed consumption and the use of nitrogen.

Consequently, an unbalanced diet with an imbalance of lysine, methionine, threonine, and partially tryptophan causes significant morphological and functional changes in the animal body.

Hemodynamic disorders in the form of vasodilation and the appearance of stasis lead to difficulties in the penetration of amino acids to the sites of protein synthesis and, as a result, to a violation of metabolic processes in parenchymal liver cells. The nature of histochemical reactions depends on the degree of dystrophic damage.

Significant fatty dystrophy and a noticeable decrease in the content of glycogen and nucleic acids were detected in the liver of animals of groups 1, 2, 3 and 4 (2 cases), along with morphological changes which led to a violation of the organ's function. Animals of the group 3 had these changes less pronounced (figures 1-4).

Figure 1. Fat inclusions in the rat’s liver of the 1st group. Sudan III staining, Volume 40, approx. 10.

Figure 2. Fat inclusions in the rat’s liver of the 2nd group. Sudan III staining, Volume 40, approx. 10.

Appearance of morphological changes in the liver, apparently, is caused by an inadequate amino acid diet and a deficiency of essential amino acids in the diet. For example, a lack of lysine disrupts the synthesis of carnitine, which plays a major role in the oxidation of fatty acids [9]. Evidently, animals of the control group, where there was a high increase in live weight, had a predominance of enzymes involved in protein synthesis over lipogenesis enzymes due to a lack of substrate (amino acids).
Figure 3. Fat inclusions in the rat’s liver of the 3rd group. Sudan III staining, Volume 40, approx. 10.

Figure 4. Fat inclusions in the rat’s liver of the 4th group. Sudan III staining, Volume 40, approx. 10.

It was found that liver obesity is primarily caused by a violation of enzyme systems, especially a decrease in the activity of enzymes that mobilize fat. Apparently, the methionine imbalance led to a disproportion between the synthesis and transport of triglycerides in group 3, as a result of a lack of phospholipids, [4].

4. Conclusion
An imbalance of amino acids in the diet of rats causes significant morphological changes in the liver, indicating a persistent violation of its structure and function. Morphological changes of liver are more pronounced in rats kept on an unbalanced diet with an imbalance of lysine, methionine and threonine, less intense – with an imbalance of tryptophan.

A balanced amino acid diet helps reduce the liver of rats.

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