# EFFECT OF DIETARY PROTEIN CONTENT ON LIVER MORPHOLOGY IN ACUTE GALACTOSAMINE POISONING

N. P. Bgatova, V. A. Shkurupii,
I. Šimek, G. N. Shorina,
Z. Červinkova, and M. Holeček

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During long-term deficiency or excess of protein in the diet of animals adaptive changes develop in the liver, which can significantly modify its resistance to xenobiotics [2] and repair processes develop in the damaged organ. Accordingly the aim of this investigation was to study the early morphologic manifestations of the response of the liver of rats adapted to diets differing in their protein content to acute poisoning with galactosamine, whose metabolites inhibit nucleic acid and protein synthesis in the liver [9].

## EXPERIMENTAL METHOD

Male Wistar rats weighing 250-300 g, divided into three groups with five animals in each group, were used. The rats of group 1 (control) received a standard diet consisting of 25% proteins, 53% carbohydrates, and 21% fats; the rats of group 2 received a low-protein diet, including 6% of proteins, 73% carbohydrates, and 21% fats; animals of group 3 received a high protein diet consisting of 60% of proteins, 27% carbohydrates, and 13% fats [7]. After 3 weeks the rats of all three groups received an intraperitoneal injection of galactosamine in 0.9% NaCl solution in a dose of 400 mg/kg. The animals were decapitated 6 and 24 h after injection of the poison. The were deprived of food for 12 h before decapitation and received only water. Samples of liver for electron microscopy were fixed in 1% $OsO_4$ solution in phosphate buffer and embedded in Epon. Ultrathin sections were studied in the JEM-100S electron microscope. Morphometry was carried out in accordance with the recommendations in [13], using test systems of squares. Differences betwen the mean values compared were considered to be significant at the $p < 0.05$ level (Student's test).

## EXPERIMENTAL RESULTS

The numerical density of free polysomal ribosomes in the hepatocytes of rats on a low protein diet was increased by 100% (Fig. 1). The concentration of membranes of the cytoplasmic organoids (in $\mu^2/\mu^3$ of cytoplasm) was reduced by 40% (Fig. 2). The surface density of the inner mitochondrial membrane was reduced by 15% and that of the other membrane by 30% (Table 1). The surface density of membranes of the smooth endoplasmic reticulum (SER) was reduced by 39% (Fig. 3). Under conditions of adaptation to any kind of factor, a decrease in the ATP concentration in the cell has been shown to lead to activation of nucleic acid and protein synthesis. During adaptation of rats to a low protein diet, similar processes evidently took place as may be shown by an increase in the number of polysomal ribosomes. However, the deficiency of exogenous amino acids under these experimental conditions probably restricted complete realization of the genetic program at the translation level. Accordingly, a twofold increase in the bulk density of the lysosomal structures due to activation of autophagocytosis (the reconstructive function of the lysosomes), evidently was adaptive in character for it could facilitate the replenishment and redistribution of the cellular reserves of amino acids [3, 6].

By the 21st day on a high protein diet complete adaptation of the hepatocytes to the high protein content in the diet had evidently taken place. The hepatocyte ultrastructure of these animals did not differ significantly from that of the control animals (Table 1, Figs. 1-3).

Central Research Laboratory, Novosibirsk Medical Institute. Department of Normal and Pathological Physiology, Medical Faculty, Karlov Univeristy, Hradec Kralove, Czechoslovakia.
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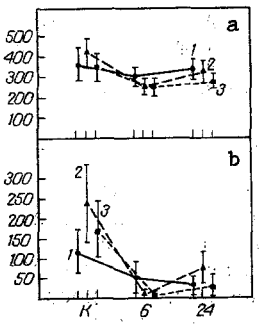


Fig. 1

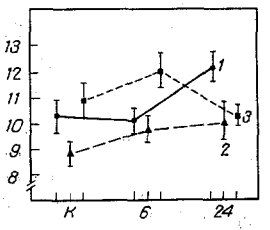


Fig. 2

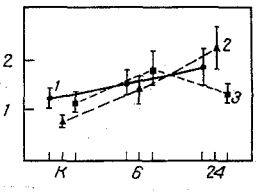


Fig. 3

Fig. 1. Results of investigation of numerical densities of ribosomes. Abscissa, time after injection of galactosamine (in h); ordinate: $N_V$) number of ribosomes in 1 $\mu^3$ of hepatocyte cytoplasm. a) Attached ribosomes; b) ribosomes in free polysomes; 1-3) standard, low-protein, and high-protein diets respectively. K) control.

Fig. 2. Results of investigation of total concentration of cytoplasmic organoid membranes. Ordinate, total surface density of membrane (in $\mu^2/\mu^3$) hepatocyte cytoplasm of SER and RER and of inner and outer mitochondrial membranes. Remainder of legend as to Fig. 1.

Fig. 3. Results of investigations of surface density of membranes of SER. Ordinate surface density (in $\mu^2/\mu^3$) of hepatocyte cytoplasm. Remainder of legend as to Fig. 1.

TABLE 1. Results of Morphometry of Hepatocyte Ultrastructures (M ± m)

| Organoids and parameters studied | Diet | Control | Time after injection of galactosamine, h | |
|---|---|---|---|---|
| | | | 6 | 24 |
| Mitochondria: | | | | |
| $V_v$ | I | 29,8±1,1 | 24,3±1,1* | 33,0±1,6 |
| | II | 22,2±1,0 | 23,6±1,1 | 27,9±1,8* |
| | III | 29,3±1,3 | 36,5±1,5* | 33,5±1,1* |
| $S_v$ | I | 1,5±0,1 | 1,4±0,1 | 1,8±0,1* |
| Outer membrane | II | 1,2±0,1 | 1,5±0,1* | 1,7±0,1* |
| | III | 1,6±0,1 | 2,1±0,1* | 1,9±0,1* |
| Inner membrane | I | 4,7±0,3 | 3,7±0,2* | 5,3±0,2 |
| | II | 4,0±0,2 | 4,0±0,2 | 4,1±0,3 |
| | III | 5,2±0,2 | 5,3±0,2 | 4,4±0,2* |
| $N_v$ | I | 0,40±0,02 | 0,38±0,02 | 0,50±0,03* |
| | II | 0,32±0,02 | 0,39±0,03 | 0,41±0,03* |
| | III | 0,44±0,03 | 0,51±0,03 | 0,43±0,02 |
| Rough endoplasmic reticulum ($S_V$) | I | 2,9±0,2 | 3,4±0,1 | 3,3±0,2 |
| | II | 2,9±0,2 | 3,0±0,1 | 2,7±0,2 |
| | III | 3,0±0,2 | 2,9±0,2 | 2,7±0,1 |
| Lysosomal structures ($V_V$) | I | 0,58±0,07 | 0,46±0,06 | 1,10±0,11* |
| | II | 1,20±0,18 | 0,52±0,09* | 0,78±0,10* |
| | III | 0,71±0,12 | 0,75±0,10 | 1,30±0,18* |
| Glycogen ($V_V$) | I | 21,0±1,4 | 4,2±0,7* | 2,4±0,6* |
| | II | 26,8±1,3 | — | 0,03±0,03* |
| | III | 22,7±1,6 | 0,4±0,2* | — |

Legend. Bulk density of structures (in % of volume of cytoplasm), $S_V$) surface density of membranes (in $\mu^2/\mu^3$ cytoplasm), $N_V$) numerical density of structures (number in 1 $\mu^3$ cytoplasm), I, II, III) standard, and low- and high-protein diet respectively. Asterisk indicates significant difference from control.

The hepatotoxicity of galactosamine is due to binding of uridine nucleotides by its metabolic products, and also to the accumulation of UDP-glucosamine, which led to inhibition of RNA and protein synthesis [9] and to a disturbance of carbohydrate metabolism [8].

The number of free polysomal and attached ribosomes 6 h after injection of galactosamine into animals on a low protein diet was 96 and 40% less respectively, whereas in animals on a high protein diet it was reduced by 94 and 30% (Fig. 1). The decrease in the number of free polysomal ribosomes in the hepatocytes of rats receiving a standard diet 24 h after injection of the poison was by 73%, but the number of attached ribosomes was unchanged (Fig. 1). When the animals were fed on a low- and high-protein diet, 6 h after injection of galactosamine glycogen had almost completely disappeared from the hepatocytes of

both groups of rats, but in animals kept on a standard diet, the maximal fall of the glycogen level was observed after 24 h (Table 1). Consequently, after injection of galactosamine, its typical effect [9] was exhibited earlier and by a greater degree in the hepatocytes of rats receiving a low- or high-protein diet. Necrotically changed hepatocytes were observed scattered diffusely throughout the lobule in animals of all three groups 6 h after injection of the poison. Their loci were larger in animals on a low-protein diet and were smallest in those on a high-protein diet. Injection of galactosamine caused the ATP concentration in the hepatocytes to fall [10]. The bulk density of the mitochondria in the hepatocytes of rats receiving a standard diet was reduced by 19% after injection of the poison, whereas the surface density of their inner membrane was reduced by 21% (Table 1). Meanwhile, the surface density of the outer mitochondrial membrane was increased by 21% in animals on the low protein diet and by 33% in those on a high protein diet. The bulk density of the mitochondria increased by 25%. Judging from these data and the numerical densities of the mitochondria, 24 h after injection of galactosamine there was an increase in the number of these organoids due to their division in animals on standard and low-protein diets. On a high protein diet the dimensions of the mitochondria were evidently increased (Table 1). Division of mitochondria is an adaptive process [5] and is a most "economic" method of increasing the metabolic rate because of the increase in surface in volume ratios. An excess of potential sources of energy (amino acids) in animals on a high protein diet, when galactosamine is injected, evidently created conditions under which there was no need to stimulate the energy-forming function of the mitochondria in this way. Moreover the mitochrondria became larger, their bulk density increased, but under these circumstances the area of the inner membrane decreased. Conversely, in animals kept on a standard or low-protein diet these processes were realized in full. The number of mitochondria was increased by 25 and 28% respectively. In the case of a low protein diet, the bulk density of the mitochondria also increased by 12%.

The content of SER (Fig. 3) was increased in the animals of all three groups after galactosamine poisoning (Fig. 3), and this was largely responsible for the increase in the total concentration of cytplasmic membranes (Fig. 2). However, this could not be the result of elevation of the level of plastic processes in the cells associated with a sharp decrease in the number of polysomal ribosomes (Fig. 1), but it was probably the result of "exposure" of SER, which previously (in the control) had been masked by glycogen.

Adaptation of the hepatocytes to diets with a low and high protein content thus endowed them with greater metabolic activity, and this led to an earlier appearance of the typical effect of galatosamine poisoning on the hepatic parenchyma than in animals on a standard diet. Meanwhile, profound inhibition of protein synthesis, which evidently continued until 24 h after injection of the poison, led to a unique "equalization" effect in the development of repair processes in the animals of all three groups. Marked hyperplasia of the intracellular structures, usually a characteristic feature of reparative regeneration, was not observed in them [13].

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