5. Yu. M. Lopukhin and S. S. Markin, Ter. Arkh., No. 1, 86 (1983).
6. N. K. Furkalo, T. I. Ivashchenko, R. M. Bol'shakova, et al., Ter. Arkh. No. 11, 119 (1982).
7. E. I. Chazov, Ter. Arkh. No. 11, 3 (1982).
8. R. A. Cooper, E.C. Arner, I. S. Willey, and S. I. Shatil, J. Clin. Invest., 55, 115 (1975).
9. L. Dintenfass, Arch. Intern. Med., 118, 427 (1967).

STRUCTURAL CHANGES IN THE LIVER PARENCHYMA OF RATS DURING LONG-TERM FEEDING ON DIETS DIFFERING IN PROTEIN CONTENT

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UDC 612.35.014.2-06:613.28:547.96

KEY WORDS: diet; liver; ultrastructure.

The liver is a metabolic center where substances taken in with the diet are transformed into a utilizable form to meet the energy and plastic requirements of the body. Individual food preferences, ecologic and economic factors determining the composition and quantity of food consumed, and also restrictions of diet in connection with disease may have a substantial influence on structural and functional parameters of the liver.

The aim of this investigation was to study the effect of diets differing in their content of food components on the structural organization of the liver.

EXPERIMENTAL METHODS

Male Wistar rats were kept for 21 days on diets of the following composition: standard (25% protein, 53% carbohydrate, 22% fat), low-protein (6% protein, 73% carbohydrate, 21% fat), high-protein (60% protein, 27% carbohydrate, 13% fat) [9]. After alkaline dissociation of samples of liver, fixed in formalin [1], films were made from cell suspensions and stained with hematoxylin and eosin; the ratio of the number of binuclear hepatocytes to the total number of hepatocytes was determined inpromille. The distribution of hepatocytes by ploidy classes were studied in liver films stained by Feulgen's method. The measurements were done on an IKÉM-1 cytophotometer [4]. Samples of liver from five animals in each group were fixed in 1% OsO_4 in phosphate buffer and embedded in Epon for electron microscopy. Epon sections 1 μ thick were stained with toluidine blue and used for morphometry, which was done in accordance with the recommendations in [11]. Differences between the mean values compared were taken to be significant at the $P < 0.05$ level (Student's test).

RESULTS

In animals kept on a low protein diet the weight of the liver decreased by 34% but its relative weight was unchanged; the number of diploid hepatocytes and the index of binuclear hepatocytes were increased (Fig. 1, Table 1). Together with an increase in the relative volume of the parenchyma, this can be taken as evidence of activation of proliferation [3]. The increased glycogen concentration (Table 2) may perhaps be connected with slowing of its phosphorylation on account of depressed glucose-6-phosphatase activity [10].

The number of attached ribosomes and the surface area of the membranes of the rough endoplasmic reticulum (RER) were slightly reduced (Fig. 2; Table 2). Mainly secretory proteins are synthesized on attached ribosomes of the hepatocyte RER [13], and under conditions of protein insufficiency their synthesis is restricted [5]. On that account, evidently, synthesis of transport proteins also was depressed, and this led to accumulation of lipids (Table 1).

Central Scientific-Research Laboratory, Novosibirsk Medical Institute. Department of Normal and Pathological Physiology, Medical Faculty, Karl University, Hradec Karlove, Czechoslovakia. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. I. Borodin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 101, No. 5, pp. 556-559, May, 1986. Original article submitted June 26, 1985.

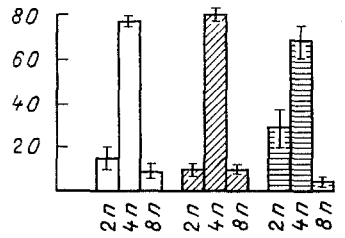

Fig. 1. Distribution of hepatocytes by ploidy. Ordinate, number of hepatocytes (in %); 2N, 4N, 8N) di-, tetra-, and octaploid hepatocytes respectively. Unshaded columns indicate standard diet, obliquely shaded, high-protein diet; horizontally shaded, low-protein diet.

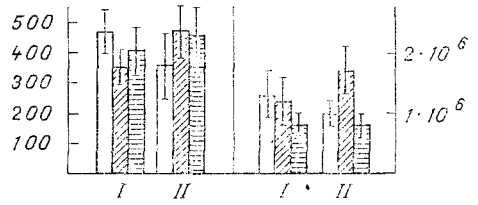

Fig. 2. Results of investigation of number of ribosomes in hepatocytes. Ordinate: on left, number of ribosome in 1 μ^3 of hepatocyte cytoplasm (N_V); on right, number of ribosomes in cytoplasm in one hepatocyte (N). I) Attached ribosomes, II) free ribosomes. Remainder of legend as to Fig. 1.

With a reduction of the intake of exogenous proteins, catabolic processes begin to predominate in the liver and the liver proteins are utilized as an endogenous source of amino acids [5]; indirect evidence of this is given by the results of investigation of lysosomes, for the fraction of secondary lysosomes was increased fivefold (Table 2).

The increase of 30% in the number of free ribosomes (Fig. 2) and also in the surface area of the membranes of RER (Table 2) could be the result of detachment of ribosomes from RER membranes [14]. The bulk density of the mitochondria was reduced by 20% and the surface area of their inner membranes also was considerably reduced (Table 2), further indirect evidence of depression of hepatocyte function.

The increase in the total number and volume of peroxisomes (Table 2) was probably linked with insufficiency of mitochondrial oxidation processes, and also with oxidation of fatty acids due to excess accumulation of lipids during feeding with a low-protein diet [12].

During the use of a high-protein diet the absolute weight of the liver increased by 60% and its relative weight by 29%; the mean volume of the hepatocytes increased by 30% (Table 1). The ploidy of the hepatocytes and the index of binuclear hepatocytes did not change significantly (Fig. 1; Table 1). Total volumes of lipid inclusions and of glycogen did not exceed the control values (Table 2). During feeding on a high-protein diet, just as on a low-protein diet, the number of attached ribosomes and the surface area of the RER membranes were somewhat lower than in the control, but the number of free ribosomes and the surface area of the RER membranes were increased (Fig. 2; Table 2).

Administration of a large quantity of protein is associated with an increase in the functional load on the hepatocytes [2]. In that case, adaptive changes might be expected in the structural organization of the hepatocytes. In fact, the total surface area of the membranes of the mitochondria and endoplasmic reticulum, both rough and smooth, was 15% greater than in

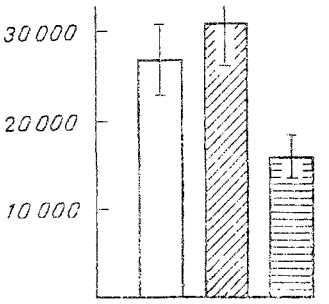

Fig. 3. Results of investigation of surface area of cytoplasmic organelles of hepatocytes (in μ^2). Legend as to Fig. 1.

TABLE 1. Volumetric and Gravimetric Parameters of Liver Hepatocytes (M ± m)

Parameter studied	Diet		
	standard	high-protein	low-protein
Weight of liver, g	8,0±0,37	12,8±0,35*	5,3±0,21*
Relative weight of liver	4,1±0,07	5,3±0,05*	3,8±0,71
Liver paranchyma (V_V)	91,1±1,0	89,1±1,85	94,0±0,80*
Hepatocytes (V)	3068,8±230,4	3815,0±204,4*	2072,3±123,9***
Index of binuclear hepatocytes	302,0±20,2	306,0±11,0	435,0±26,0*

Note. V_V) Fraction by volume; V) volume (in μ^3). *) Difference from control is significant; ***) [not explained in Russian original – Editor].

TABLE 2. Results of Morphometry of Subcellular Structures of Hepatocytes (M ± m)

Patameter studied	Diet		
	standard	high-protein	low-protein
Mitochondria (V_V)	31,1±0,96	32,0±0,97	15,0±0,95***
Mitochondria:			
outer membrane (S_V)	1,4±0,04	1,5±0,05	1,2±0,05**
inner membrane (S_V)	5,0±0,18	4,2±0,17**	4,4±0,22*
Lysosomes $\left(\frac{V_V \text{ of secondary}}{V_V \text{ of primary}}\right)$	1,9±0,76	0,9±0,36	5,0±1,18
RER (S_V)	2,4±2,0	2,0±0,2	2,1±0,1
Smooth endoplasmic reticulum (S_V)	0,8±0,11	0,9±0,09	1,0±0,13
Peroxisomes			
V_V	1,5±0,16	1,7±0,13	1,9±0,16
N_V	0,1±0,01	0,14±0,01*	0,18±0,02***
Lipid inclusions			
V_V	0,28±0,28	5,02±3,26	139,6±27,67***
V_V	0,01±0,1	0,14±0,09	7,5±1,4***
Glycogen			
V_V	389,4±39,0	466,6±46,79	595,8±49,57**
V_V	13,8±0,8	13,6±0,75	32,1±1,6***

Note. V_V) Bulk density of structures (in % of volume of cytoplasm), S_V) surface density (in μ^2/μ^3 volume of cytoplasm), V) total volume of structures (in μ^3, calculated per hepatocyte), N_V) numerical density of structures (number of 1 μ^3 volume of cytoplasm). [Significance of asterisks not explained in Russian original – Editor.]

the control (Fig. 3). However, the bulk density of the mitochondria corresponded to the control value, whereas the surface density of the inner membranes was reduced by 16% (Table 2). The decrease in surface area of the inner mitochondrial membranes could be the result of inhibition of some of the enzymes of glycolysis and of the Krebs' cycle in connection with accumulation of surplus energy in the form of an increase in the intracellular ATP concentration during consumption of a diet with a high protein content [6].

The need to "reprocess" the surface energy mainly with a view to its long-term accumulation in the form of high-molecular-weight compounds [6], evidently was responsible for the increase in bulk and numerical density of peroxisomes (Table 2).

During consumption of a low-protein diet, besides evidence of adaptive structural changes in the liver parenchyma (proliferation of hepatocytes, an increase in the number of secondary lysosomes, undertaking mobilization and redistribution of endogenous reserves) [7], which evidently took place in the early period of consumption of the diet, atrophic changes thus also were observed. These latter evidently predominated in the later periods of consumption of the low-protein diet by the animals. If the protein intake was excessive, on account of the increased functional load on the liver, adaptive structural changes in its parenchyma were expressed as hypertrophy of hepatocytes and hyperplasia of their subcellular structures.

LITERATURE CITED

1. V. Ya. Brodskii, N. N. Tsireksedze, M. E. Kogan, et al., Tsitologiya, 25, 260 (1983).
2. V. A. Konyshev, Diet and Regulating Systems of the Organism [in Russian], Moscow (1985).
3. B. N. Kudryavtsev, M. B. Kudryavtseva, E. É. Zavadskaya, et al., Tsitologiya, 24, 431, (1982).
4. É. M. Kurashov, Tsitologiya, 18, 1406 (1976).
5. N. N. Lapteva, The Pathophysiology of Protein Metabolism [in Russian], Moscow (1970).
6. L. F. Panchenko, A. M. Gerasimov, and V. D. Antonenkov, The Role of Peroxisomes in Cell Pathology [in Russian], Moscow (1981).
7. A. A. Pokrovskii and V. A. Tutel'yan, Lysosomes [in Russian], Moscow (1976).
8. Z. A. Ryabinina and V. A. Benyush, Polyploidy and Hypertrophy of Cells in Growth and Restoration Processes [in Russian], Moscow (1973).
9. P. Fabry, Čsl. Fysiol., 8, 529 (1959).
10. K. Fletcher, Amer. J. Clin. Nutr., 19, 170 (1966).
11. A. V. Loud, J. Cell Biol., 37, 27 (1968).
12. P. B. Lazarow, in: The Liver: Biology and Pathobiology, New York (1982).
13. M. M. Mucekler and H. C. Pitot, J. Cell Biol., 90, 495 (1981).
14. D. S. Sarma, E. Verney, and H. Sydransky, Lab. Invest., 27, 48 (1972).

EFFECTIVENESS OF COLCHICINE IN EXPERIMENTAL ALCOHOL-INDUCED LIVER DAMAGE

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UDC 616.36-004.4-085.277.3:
547.944.6]-035.1

KEY WORDS: liver; alcohol-induced damage; colchicine.

An important role in the pathogenesis of the toxic action of alcohol on the liver is played by disturbances of a number of enzymic and metabolic processes in hepatocytes. Induction of mono-oxygenases and mobilization of cytochrome P-450-dependent hydroxylation reactions (including the microsomal ethanol-oxidizing system), initiation of free-radical reactions and processes of lipid peroxidation (LPO), as well as activation of mesenchymal reactions (collagen synthesis, fibrillogenesis), deserve attention in this context [3].

The first two mechanisms are provided by a single NADPH-dependent electron and proton transport system in membranes of the endoplasmic reticulum (ER) of the hepatocytes [1]. Under the influence of oxidation of ethanol, conditions are created in ER for intensification of free-radical reactions on induced cytochrome P-450 and an increase in the activity of peroxidation of cell membrane phospholipids [2, 9, 11]. As a result of enzymic and metabolic modifications in the hepatocytes under the influence of ethanol mesenchymal reactions are activated.

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 101, No. 5, pp. 559-562, May, 1986. Original article submitted April 25, 1985.

0007-4888/86/0005-0610$12.50