EFFECT OF A LOW PROTEIN DIET ON RESTORATION OF THE RAT LIVER PARENCHYMA AFTER CARBON TETRACHLORIDE POISONING

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Regeneration of an organ after injury is accompanied by a raised level of plastic processes in the cells [7]. As a result of this, the optimal protein content of the diet is one factor which determines the effectiveness of structural and functional compensation of the lost part of the cell population. The repair process in the liver parenchyma takes place through division and hypertrophy of hepatocytes [1].

The aim of the present investigation was accordingly to study manifestations of intracellular repair processes in the hepatic parenchyma after toxic damage in animals kept on a low protein diet.

EXPERIMENTAL METHOD

Male Wistar rats weighing 190-200 g were divided into two groups. The animals of group 1 (control) were kept on a standard diet consisting of 25% protein, 53% carbohydrate, and 21% fat; rats of group 2 were kept for 5 weeks on a low protein diet consisting of 6% protein, 73% carbohydrate, and 21% fat. The diets were balanced with respect to calorific value and were made up in accordance with the recommendations in [8]. After 3 weeks on these diets, rats of both groups were given an intraperitoneal injection of carbon tetrachloride (CCl_4) in a dose of 0.075 ml/100 g body weight in 0.2 ml of olive oil, under superficial ether anesthesia. The animals were decapitated between 9:30 a.m. and 10 a.m., 6 h and 1, 2, 3, and 14 days after administration of the poison. Intact rats receiving standard and low protein diets for 21 days served as the control. Before decapitation the rats were deprived of food for 12 h but were given free access to water. After alkaline dissociation of specimens of liver fixed in formalin [3], the ploidy of the hepatocytes was investigated in films prepared from a cell suspension, stained by Feulgen's method, by means of the IKÉM-1 cytophotometer [5], and the average ploidy (the number of haploid amounts of DNA in an average hepatocyte) was calculated in accordance with recommendations in [2, 6]. Specific DNA activity in the hepatocyte nuclei was determined according to [10] 1 h after intraperitoneal injection of ^{14}C-thymidine (specific activity 44 mCi/mmole) in a dose of 0.6 mCi/100 g body weight. Liver samples for electron microscopy were taken from five rats of group 1 and five rats of group 2 at each period of investigation. The liver was fixed in 1% OsO_4 solution in phosphate buffer and embedded in Epon. Sections 1 μ thick were cut from these specimens, stained with toluidine blue, and used for morphometry. Ultrathin sections were examined in the JEM 100S electron microscope. Morphometry of semithin and ultrathin sections was carried out in accordance with recommendations in [11]. Differences between the mean values were considered significant at the $p < 0.05$ level and were assessed by Student's test.

EXPERIMENTAL RESULTS

Keeping the animals on a low protein diet for 3 weeks led to a decrease of 32% in the volume of the hepatocytes (Table 1) and a decrease of 14% in the average ploidy (Fig. 1b). A decrease also was observed in the total surface area of membranes of the rough endoplasmic reticulum and of the inner and outer mitochondrial membranes by 42% (Fig. 1d) and the total volume of the lipid inclusions was increased (Table 1). All these observations, together

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TABLE 1. Results of Morphometry of Hepatocytes (M ± m)

Test object	K	Time after injection of CCl_4				
		6 h	1 day	2 days	3 days	14 days
Volume of hepatocytes (V)	3069±230	4212±460*	4662±530*	5285±537*	5028±356*	5244±465*
	2072±124	2906±168*	3686±310*	3868±324*	3648±266*	3960±323*
Nucleo-cytoplasmic ratio	0,06±0,01	0,07±0,01	0,07±0,01	0,07±0,01	0,07±0,01	0,06±0,01
	0,09±0,01	0,1±0,01	0,08±0,01	0,1±0,01	0,09±0,01	0,09±0,01
Mitochondria:						
inner membrane (S_V)	5,0±0,18	6,0±0,25*	4,7±0,24	3,2±0,15*	2,9±0,15*	3,4±0,16*
	4,4±0,22	4,8±0,25	2,6±0,17*	2,6±0,17*	2,8±0,15*	3,8±0,18*
outer membrane (S_V)	1,4±0,04	1,4±0,08	1,6±0,06*	0,6±0,02*	1,3±0,09	1,5±0,08
	1,2±0,05	1,7±0,08*	1,1±0,05	0,5±0,04*	1,2±0,06	1,3±0,08
Concentration of membranes (ΣS_V)	9,5±0,26	11,5±0,31*	9,9±0,31	7,2±0,22	8,4±0,29*	8,2±0,25*
	8,7±0,30	10,2±0,33*	7,2±0,51*	7,7±0,23*	7,1±0,28*	8,4±0,27
Lipid inclusions (V_V)	0,01±0,01	0,6±0,23*	2,0±0,72*	1,2±0,47*	1,3±0,81*	0,3±0,13*
	7,5±1,40	4,2±1,12	6,1±1,20	2,5±0,75*	1,1±0,42*	1,9±0,59*

Legend. Numerator — standard diet; denominator — low protein diet. V) Volume, in μ^3; S_V) surface density (in μ^2/μ^3 volume of cytoplasm); V_V) bulk density of structures (in % of volume of cytoplasm). Values differing significantly from those in control (K) are indicated by an asterisk.

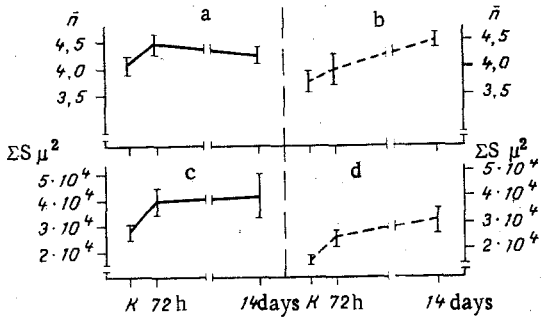

Fig. 1. Results of investigation of average ploidy and total surface area of organoid membranes, calculated per hepatocyte. n̄) Average ploidy of hepatocytes; ΣS) total surface area of rough endoplasmic reticulum and inner and outer mitochondrial membranes; a, c) group 1; b, d) group 2. Here and in Figs. 2 and 3: abscissa: K) control; time after injection of CCl_4.

with an increase of 1.5 times in the nucleo-cytoplasmic ratio of the hepatocytes (Table 1) are evidence of atrophy in the liver parenchyma.

The volume of the hepatocytes was increased by 40 and 27% respectively (Table 1) 6 h after injection of CCl_4 in rats kept on standard and low protein diets. In the animals receiving a low protein diet the concentration of organoid membranes (in μ^2/μ^3 cytoplasm) was increased by 17%, compared with 20% in animals receiving the standard diet (Table 1). In rats receiving the low protein diet specific DNA activity was 78% higher than in the control group (Fig. 2). These findings are evidence of the early development of repair processes in the organ, and the effect was more marked in animals kept on a low protein diet.

Specific DNA activity 48 h after injection of CCl_4 was twice as high in rats kept on a low protein diet as in animals of the control group, and it fell to the initial level after 72 h (Fig. 2).

In the animals of both groups centrilobular necrosis was observed 24 h after CCl_4 poisoning. At this time, in the rats receiving a low protein diet, the number of free polysomal ribosomes reached a maximum (Fig. 3), and this increase in their number preceded the peak of DNA synthesis, a characteristic feature of the prereplicative period [4]. Meanwhile, a smaller increase in the number of free polysomal ribosomes was found in the liver of the animals kept on a standard diet (Fig. 3) and their raised level of DNA synthesis continued

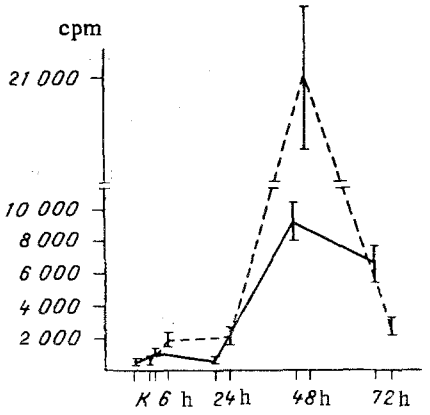

Fig. 2

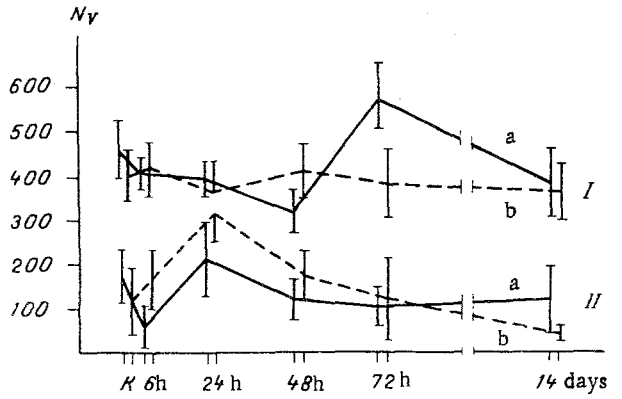

Fig. 3

Fig. 2. Results of investigation of specific DNA activity.

Fig. 3. Numerical density of ribosomes. N_V) Number of ribosomes per cubic micron of hepatocyte cytoplasm; I) attached ribosomes, II) free polysomal ribosomes. Remainder of legend as to Fig. 1.

until 72 h after injection of CCl_4 (Fig. 2). The number of attached ribosomes was increased at this period in rats of the control group (Fig. 3), evidence of a raised level of synthesis "for export" [9]. Meanwhile, in the animals kept on a low protein diet, the number of attached ribosomes remained unchanged throughout the experiment (Fig. 3).

The end result of replicative DNA synthesis may be entry of the cells into mitosis or an increase in ploidy of the hepatocytes, i.e., realization of manifestations of repair processes characteristic of the liver [2] after injury to the organ. The greater increase in ploidy of the hepatocytes (14 days after injection of CCl_4) was observed in the regenerating liver of animals receiving a low protein diet (Fig. 1b). However, despite the higher level of ploidy of the hepatocytes than in the control group, the cell volume in these animals was 45% less (Table 1), the total surface area of membranes of the rough endoplasmic reticulum and the inner and outer mitochondrial membranes was 23% less (Fig. 1d) and the numerical density of the free polysomal ribosomes was five times less (Fig. 3), evidence of atrophy in the cytoplasm. Meanwhile the number of attached ribosomes was not reduced (Fig. 3). This can be taken as evidence that despite the atrophic changes in the hepatocytes, the external secretory functions of the liver may be preserved at the basal level.

Thus in the early periods of development of repair processes in animals on a restricted protein diet the increase in DNA synthesis was accompanied by a raised level of intracellular processes. Despite increased ploidy of the hepatocytes, atrophic changes developed in the cytoplasm, evidence of reduced ability of the cells to realize their genetic program in the period of regeneration on account of the protein deficiency, i.e., evidence of inadequate restoration of the structure and function of the organ.

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SEASONAL FLUCTUATIONS IN ACTIVITY OF HUMAN BONE MARROW STROMAL PRECURSOR CELLS

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Circadian and circennial changes in physiological processes in man and animals have recently been described, and in particular, seasonal changes have been shown to take place in the peripheral blood leukocyte count, activity of adrenocortical and sex hormones, and levels of certain vitamins [2, 3]. Exacerbations of rheumatic fever and rheumatoid arthritis in the spring and fall were first observed long ago. However, there are no data on seasonal changes in activity of cells possessing stem qualities, which include stromal precursor cells (CFU-F) of the bone marrow, which play an important role in bone regeneration and in the formation of the hematopoietic microenvironment [5, 6].

The aim of this investigation was to study CFU-F activity of human bone marrow in the course of the year, based on the results of cloning in vitro.

EXPERIMENTAL METHOD

The cloning efficiency of bone marrow CFU-F was investigated in 250 orthopedic patients over a period of observation lasting 7 years: from February, 1979, through July, 1985. The results of cloning of CFU-F from the bone marrow of the sternum, iliac crest, tibial tuberosity, and other parts of the skeleton in patients with lesions of the bones and joints (osteomyelitis, aseptic necrosis, pseudarthrosis, arthritis deformans, congenital dislocation of the hip, and so on), but with no evidence of degenerative, dystrophic, or inflammatory changes at the site from which the material was obtained, were subjected to analysis. Under these circumstances the results could be pooled and analyzed together.

Cloning of CFU-F was carried out in 73 cases without a feeder and in 177 cases with a rabbit feeder by the method described in [1]. Culture was carried out in glass flasks (area of bottom 69 and 85 cm^2) in medium 199 with 15-20% human group AB (IV) serum for 12-14 days. The growing colonies were fixed with ethanol and stained by the Romanovsky–Giemsa method. Cloning efficiency was determined by the number of growing colonies per 10^5 transplanted human nucleated bone marrow cells. Concentrations of fibroblast-like cells containing at least 50 cells were taken to be colonies [6]. The results were subjected to statistical analysis by nonparametric tests [4].

EXPERIMENTAL RESULTS

The results of analysis of cloning of human CFU-F month by month for a year are given in Table 1.

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