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STRUCTURAL TRANSFORMATIONS IN HEPATOCYTES FOLLOWING ADMINISTRATION OF RHEOPOLYGLUCIN TO MICE AND SUBSEQUENT EXPOSURE TO STRESS

V. A. Shkurupii

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The introduction of various substances which are metabolized in the liver into the animal body is one way of increasing the function load on that organ. This gives rise to intensification of plastic processes in the hepatocytes [9, 11], to the creation of a structural basis for increased liver function, and intensification of repair processes in the damaged organ [2, 3, 9]. Administration of the lysosomatropic agent rheopolyglucin to mice accelerated resorption of necrotic foci and restoration of the parenchyma of the liver after its damage by carbon tetrachloride [6].

The aim of this investigation was to study structural changes taking place in hepatocytes after administration of rheopolyglucin, which is metabolized in the liver, and the particular features of the response of its parenchyma to stress.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA mice aged 2 months and weighing 19-21 g. The animals in group 1 (control) were superficially anesthetized with ether and given an injection of 0.9% NaCl solution into the caudal vein in a dose of 1 ml/100 g body weight. Mice of group 2 received a similar injection of rheopolyglucin (10% solution of partially hydro-

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

Parameter studied	Control	Experiment	
		rheopolyglucin	rheopolyglucin + stress
Nucleus of hepatocyte ($\bar{V}$)	237,0±7,58	337,4±10,8*	349,4±9,68*
Cytoplasm of hepatocyte ($\bar{V}$)	2398,4±239,0	3510,4±314,0*	3469,5±125,4*
Hepatocyte ($\bar{V}$)	2635,4±240,0	3847,8±316,4*	3818,9±125,8*
Ratio of number of binuclear hepatocytes to total number of hepatocytes	0,041±0,007	0,062±0,009	0,080±0,012*

Legend. $\bar{V}$) Volume (in μ^3). Here and in Table 2: *p < 0.05 compared with control.

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TABLE 2. Results of Morphometry of Hepatocyte Ultrastructures (M ± m)

Parameter and ultrastructure studied	Control	Experiment	
		rheopolyglucin	rheopolyglucin + stress
Lysosomal structures (V_V)	0,89±0,137	1,30±0,150*	1,10±0,145
Autophagic vacuoles (V_V)	0,43±0,09	0,93±0,19	0,60±0,11
Mitochondria (V_V)	17,5±0,85	20,1±0,81*	23,0±0,93*
Mitochondria, internal membrane (S_V)	3,40±0,19	4,01±0,24*	4,59±0,24*
Mitochondria (N_V)	0,22±0,01	0,19±0,01*	0,16±0,01*
RER (V_V)	8,6±0,62	11,1±0,66*	10,8±0,68*
RER (S_V)	2,93±0,20	3,41±0,23	3,48±0,21*
Glycogen (V_V)	11,3±1,18	9,2±1,12	6,4±1,86*
Lipid inclusions (V_V)	5,6±0,84	3,6±0,54	4,7±0,67
Ribosomes:			
free (N_V)	320±27	347±27	267±20
attached (N_V)	147±13	213±19*	213±17*

Legend. V_V) Bulk density (in % of volume of cytoplasm). S_V) Surface density of membranes (in μ^2/μ^3 volume of cytoplasm). N_V) Numerical density (number of ribosomes in 1 μ^3 of cytoplasm).

lyzed dextran with molecular weight of 30-40 kD) in a dose of 1 ml/100 g body weight. Mice of groups 1 and 2 were decapitated 2 h after the procedures. Mice of group 3 received rheopolyglucin by the same method and in the same dose as those of group 2. These mice were subjected to stress 2 h later by keeping them on the AVB-4P shaker once for 45 min, with a frequency of 2.5 Hz. Samples of liver were obtained immediately after the end of exposure. Each group contained five mice, all with free access to food and water. Specimens of liver were fixed in 1% OsO_4 solution in phosphate buffer and embedded in Epon. Sections 1 μ thick were cut from these blocks, stained with toluidine blue, and used for hepatocyte morphometry. Ultrathin sections were stained with a saturated aqueous solution of uranyl acetate and with lead citrate and photographed in the JEM 100S electron microscope with a magnification of 7000. Morphometry was carried out on the negatives. During morphometry the recommendations put forward previously were used for guidance [11] and test systems of squares were employed. Differences between the mean values compared were considered to be significant at the $p < 0.05$ level (Student's test).

EXPERIMENTAL RESULTS

The volume of the nuclei was increased by 42% and the volume of the cytoplasm of the hepatocytes by 47% 2 h after injection of rheopolyglucin (Table 1). Investigation of the liver specimens in the electron microscope revealed characteristic changes in the lysosomes in connection with accumulation of rheopolyglucin in them [6]. The number of primary lysosomes was reduced whereas the number of secondary was increased. Consequently, and with a small increase in the bulk fraction of autophagic vacuoles, the volume of the lysosomal system was increased by 46% (Table 2). The bulk density of the mitochondria was increased by 28% and, together with a reduction of 14% in the number of these organoids (Table 2), this was evidence of their fusion. The surface area of the inner mitochondrial membrane was increased by 18% (Table 2). Allowing for the increase in volume of the hepatocyte cytoplasm, the total surface area of the mitochondrial membranes — both inner and outer, and of the endoplasmic reticulum, peroxisomes, and lysosomal structures — in one hepatocyte was increased by 58.7% and the number of attached by 112% (Fig. 2). All these data are evidence of intensification of intracellular plastic processes, whereas the sharp increase in the number of adherent ribosomes is evidence of intensification of external secretory function.

According to data in the literature [11] hyperplasia of the intracellular structures in the hepatocytes and an increase in weight of the liver [9] were observed after injection of phenobarbital, which is metabolized by hepatocytes, into the rats. Hyperplastic processes in the liver were observed after administration of sucrose to animals [7]. It was also shown that injection of dextran into animals was accompanied by a more than twofold increase in acid phosphatase activity [8, 10]. Rheopolyglucin accumulating in lysosomes of liver cells is known to undergo hydrolysis to glucose [1]. On the basis of the facts described above it can be tentatively suggested that intensification of plastic processes in the hepatocytes after injection of rheopolyglucin was due to an increased functional load on account of its hydrolysis.

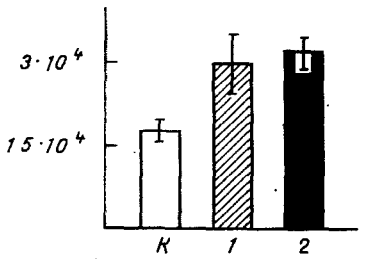

Fig. 1

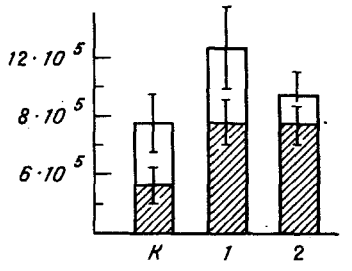

Fig. 2

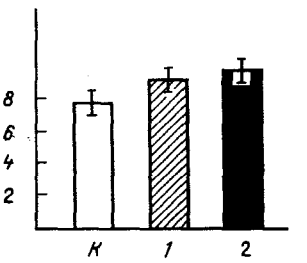

Fig. 3

Fig. 1. Total surface area of membranes of cytoplasmic organoids of hepatocytes (in μ^2 calculated per hepatocyte). K) Control; 1) 2 h after injection of rheopolyglucin; 2) 2 h after injection of rheopolyglucin followed by exposure to stress for 45 min.

Fig. 2. Results of determination of number of ribosomes (number of ribosomes in one hepatocyte). Unshaded columns — free ribosomes; obliquely shaded — attached ribosomes. Remainder of legend as to Fig. 1.

Fig. 3. Total surface density of membranes of cytoplasmic organoids of hepatocytes (surface area of membranes in μ^2 in 1 μ^3 volume of cytoplasm. Legend as to Fig. 1.

It was shown previously [4] that exposure of CBA and C57BL/6 mice to stress by a method similar to that described above was accompanied by a sharp increase in the blood concentration of 11-hydroxycorticosteroid hormones and by the development of catabolic processes in the hepatocytes. Glycogen disappeared from the hepatocytes, the number of free ribosomes and ribosomes attached to membranes of the rough endoplasmic reticulum (RER) was sharply reduced, and processes of autophagocytosis and division of the mitochondria were activated [4].

Meanwhile exposure of animals previously treated with rheopolyglucin to stress was not accompanied by intensification of catabolic processes in the hepatocytes. Conversely, an increase in the number of binuclear hepatocytes and in the volumes of the hepatocyte nuclei and cytoplasm was observed (Table 1). The bulk density of the mitochondria was increased by 31% and, in conjunction with the decrease of 23% in their numerical density, indicates fusion of these organoids (Table 2). The volume of the RER was increased by 26% and its surface area by 19% (Table 2). The total concentration of membranes (in μ^2/μ^3 cytoplasm) of the organoids was increased by 24% (Fig. 3), their total surface area per cell was increased by 74% (Fig. 1), and the number of free ribosomes was increased by 21% and of attached ribosomes by 110% (Fig. 2). The bulk densities of the lysosomal apparatus and autophagic vacuoles did not exceed the control values (Table 2). Thus exposure to stress under these conditions did not lead to the development of catabolic processes in the hepatic parenchyma, evidently because preliminary injection of rheopolyglucin led to the creation of a structural basis for the formation of an adequate cell response to the functional demands made on the animal without activation of the "expensive" mechanism of adaptation, namely autophagocytosis [5].

The ability of rheopolyglucin to stimulate repair processes in the liver after its injury, together with the data described in this paper, can be regarded as a basis for the widening of indications for clinical use of rheopolyglucin. The results indicate possible ways of prevention and correction of pathological states with the aid of lysosomotropic agents.

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CELL COMPOSITION OF SUBCUTANEOUS CONNECTIVE TISSUE AFTER SINGLE AND REPEATED GENERAL OVERHEATING

V. V. Vinogradov, N. S. Permitina, and N. F. Vorob'eva

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Generalized overheating leads to morphological and functional changes in the cardiovascular and respiratory systems and in the kidneys and liver [2-5]. During and after hyperthermia the content of lipid peroxidation products, which exert a toxic action [6], increases in all the organs and in the blood. The increase in blood levels of biologically active substances and also of products of incomplete metabolism [4] gives rise to autointoxication.

The aim of this investigation was to study the cell composition of subcutaneous connective tissue (SCCT) in the early and late stages after single and repeated general overheating.

EXPERIMENTAL METHOD

Experiments were carried out on 198 noninbred male rats weighing from 170 to 200 g. The animals were kept on a standard diet with free access to water. The rats were subjected to a single period of overheating for 1 h or to three separate periods (45 min every 3 days) at 43.5°C in a ventilated heat chamber. Animals kept at 20°C served as the control. The rats were decapitated immediately and 1, 3, 7, 11, 15, 30, and 62 days after a single period of overheating and 6, 7, 9, 13, 17, 21, 36, and 68 days after the initial period of repeated overheating. The cell composition of SCCT was studied in intervascular regions of film preparations by the random samples method. The leukocyte formula of the peripheral blood was determined. After a single overheating, the biological toxicity of the blood serum was estimated by an express method (the paramecium test).

EXPERIMENTAL RESULTS

The conditions of overheating chosen were extremal and in some cases death ensued. Mortality depended on the duration of exposure: 20.7% after an exposure of 1 h and 1.3% after an exposure of 45 min. On repetition of overheating, the number of fatal cases increased: 1.3% after the first exposure, 7.7% after the second, and 13.9% after the third. Single and repeated whole-body overheating led to dehydration: the animals lost about 6% of their total body weight after a single overheating lasting 1 h, and up to 4% after overheating for 45 min. The body weight was restored 3 days later. The survival rate of the animals after exposure to a high temperature is evidently determined by individual features of the adaptive capacity of the physiological systems and also by the duration and repetitiveness of the overheating.

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