Clasmatosis of mitochondrial fragments, which in each of the cases described above under normal conditions is also observed during very intensive utilization of energy by the cell, is very interesting.

Thus clasmatosis of their fragments in the cell and its organelles takes place whenever it is necessary to supply the organism or the cell quickly with certain metabolic products or to remove something from the cell quickly, as in the case of the erythroblast; in other words, it is one of the mechanisms of adaptation of the cell and its organelles to changing environmental conditions.

LITERATURE CITED

1. A. P. Avtsyn and V. A. Shakhlamov, The Ultrastructural Basis of Cell Pathology [in Russian], Moscow (1979), p. 92.
2. T. P. Beketova and S. M. Sekamova, Byull. Éksp. Biol. Med., No. 7, 107 (1975).
3. M. M. Kalashnikova, Byull. Éksp. Biol. Med., No.7, 84 (1979).
4. M. M. Kalashnikova, Byull. Éksp. Biol. Med., No. 5, 620 (1981).
5. M. M. Kalashnikova, in: Ultrastructural Pathology of the Liver [in Russian], Riga (1984), pp. 65-72.
6. A. N. Studitskii, Evolutionary Morphology of the Cell [in Russian], Moscow (1981), pp. 102 and 108.
7. K. Cowan and J. Sargent, in: Bioenergetics and Growth of Fishes [Russian translation], Moscow (1983), pp. 8-61.
8. A. Policard and C. A. Baud, Les Structures Inframicroscopiques Normales et Pathologiques des Cellules et des Tissus, Masson, Paris (1958).
9. P. Stöhr and W. H. W. von Möllendorff (ed.), Lehrbuch der Histologie und der pathologischen Anatomie des Menschen, 23rd edn., Fischer, Jena (1933).

TIME COURSE OF CHANGES IN RAT HEPATOCYTE ULTRASTRUCTURE AFTER HEPATIC ISCHEMIA

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KEY WORDS: hepatic ischemia; hepatocyte ultrastructure; morphometry.

Temporary exclusion of the liver from the circulation during operations is attended by the risk of irreversible changes in the hepatocytes. There is as yet no general agreement on the optimal time for which the organ can be excluded from the circulation [1, 2, 4, 5] while preserving its function. Hepatic ischemia, however, (caused total extirpation of the organ before transplantation, compression of the main vessels during operations on the "dry" liver, embolism, thrombosis, atherosclerosis, and so on), leads to structural changes in the organ [1, 2, 4, 5]. In this connection it is useful to have a clear idea of the character of the changes, especially subcellular, taking place in cells of the liver after its exclusion from the circulation.

The aim of this investigation was to study the subcellular organization of rat hepatocytes during and at various times after ischemia of the liver.

EXPERIMENTAL METHOD

Forty male Wistar rats weighing 150-180 g were used. For 12-14 h before the experiments the rats were deprived of food but had free access to water. Intact animals (group 1) served as the control. The operation consisted of compression of the hepato-duodenal ligament after preliminary isolation of the bile duct under intraperitoneal pentobarbital anesthesia (40

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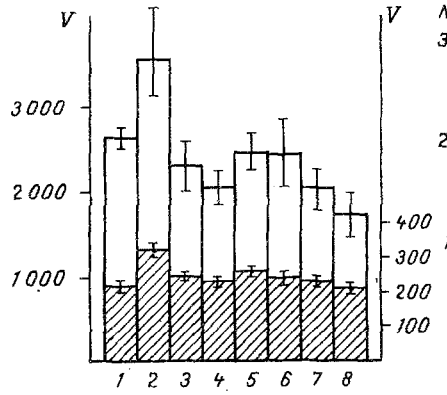

Fig. 1

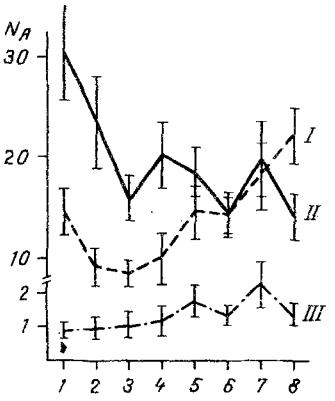

Fig. 2

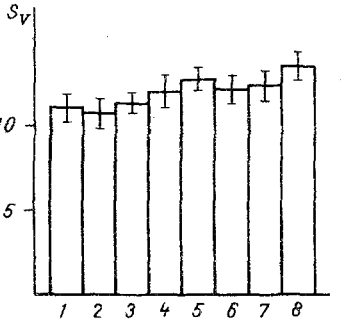

Fig. 3

Fig. 1. Results of investigation of volume of hepatocytes (unshaded columns) and their nuclei (shaded columns). Abscissa, groups of animals: 1) intact (control), 2) 30 min of ischemia, 3-8) after 2, 12, and 24 h and 3, 7, and 14 days of recirculation respectively. Ordinate: on left — mean volume (V) of hepatocytes, on right — mean volume of their nuclei (in μ^3).

Fig. 2. Results of investigation of number of free ribosomes (I), attached ribosomes (II), and polysomes (III) in 1 μ^2 area of section of cytoplasm of rat hepatocyte. Ordinate, number of structures (N_A) in 1 μ^2 of cytoplasm. Remainder of legend as to Fig. 1.

Fig. 3. Results of investigation of total area of membranes of cytoplasmic organelles in rat hepatocytes. Ordinate, total surface area (S_V) of cytoplasmic membranes: of outer and inner mitochondrial membranes, membranes of rough and smooth endoplasmic reticulum (in μ^2/μ^3 of cytoplasm). Remainder of legend as to Fig. 1.

mg/kg body weight). The rats were decapitated at the 30th minute of normothermic ischemia before removal of the clamp, and after 2, 12, and 24 h, and 3, 7, and 14 days of revascularization (animals of groups 2, 3, 4, 5, 6, 7, and 8, respectively).

At each stage of the investigation pieces of liver from five animals were fixed in 1% OsO_4 solution in phosphate buffer and embedded in Epon. Semithin sections were then cut to a thickness of 1 μ, stained with toluidine blue, and used to determine the volumes of the hepatocytes and their nuclei. Ultrathin sections were stained with an aqueous solution of uranyl acetate and with lead citrate, and examined in the JEM-100S electron microscope. In sections prepared from each animal 20 areas of cytoplasm of the hepatocytes, chosen by random sampling, were photographed and the pictures were studied morphometrically according to recommendations in [6]. Differences between means were considered to be significant at the $P < 0.05$ level (by Student's test).

EXPERIMENTAL RESULTS

Hepatic ischemia for 30 min was not followed by necrotic changes in the hepatocytes, but led to a marked increase in volume of the hepatocytes and their nuclei (Fig. 1, group 2). The matrix of the mitochondria was translucent in the hepatocytes of these animals, the relative volume of the mitochondria was increased (Table 1), but their number and the surface area of their outer membrane were indistinguishable from the control value, evidence of swelling of the mitochondria. At this stage the number of free and attached ribosomes was reduced (Fig. 2, group 2), and cisterns of the rough endoplasmic reticulum (RER) were narrowed (Table 1). According to some workers [5], swelling of the cisterns of the RER in response to hepatic ischemia is accompanied by an increase in volume of the cisterns of RER. However, the morphometric data given in Table 1 indicate a persistent decrease in volume of the cisterns of RER at the 30th minute of hepatic ischemia. Similar changes in volume of the cisterns of RER also were observed after removal of the clamp, except after 12 h of recirculation in the liver (Table 1).

In the postischemic period the volumes of the hepatocytes and their nuclei changed, but on the whole the dominant tendency was for the parameters to decrease (Fig. 2, groups 3-8).

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

Parameter studied	Control	30 min of ischemia	Time after removal of clamp from hepato-duodenal ligament					
			2h	12 h	24 h	3 days	7 days	14 days
Mitochondria (Vv)	22,7±0,88	26,8±0,99*	33,7±0,96*	27,3±1,23*	25,5±0,84*	29,3±0,97*	27,2±1,14*	26,7±0,99*
Mitochondria, outer membrane (Sv)	1,22±0,04	1,29±0,04	1,8±0,05*	1,55±0,06*	1,44±0,05*	1,5±0,07*	1,5±0,05*	1,42±0,05*
Mitochondria, inner membrane (Sv)	4.9±0,35	5,2±0,34	5,3±0,21	5,7±0,37	6,0±0,33*	6,22±0,3*	6,4±0,38*	7,46±0,4*
Mitochondria (Nv)	0,32±0,02	0,29±0,02	0,46±0,02*	0,39±0,03	0,33±0,02	0,33±0,02	0,37±0,04	0,31±0,02
	19,2±0,95	13,1±0,90*	14,2±0,64*	17,2±0,84	16,5±0,67*	16,5±0,63*	11,9±0,72*	15,5±0,84*
RER (Vv)	4,82±0,24	4,2±0,30	4,2±0,13*	4,63±0,24	5,2±0,20	4,31±0,18	4,73±0,28	4,77±0,19
Lysosomal structures (Vv)	0,04±0,01	0,04±0,01	0,1±0,03*	0,04±0,01	1,0±0,23	0,1±0,02	0,09±0,03	0,12±0,02*
Lipid inclusions (Vv)	0,45±0,15	0,9±0,25	1,1±0,35	1,34±0,31*	4,15±0,87*	2,28±0,85*	1,68±0,61*	1,71±0,4*
Glycogen (V_V)	16,3±1,16	17,5±0,95	13,2±0,42*	19,5±1,05*	15,5±0,5	16,8±0,43	17,4±1,22	13,9±0,4*

Legend. V_V) Bulk density of ultrastructures (in per cent of volume of cytoplasm); S_V) surface density of membranes of ultrastructures (in μ^2/μ^3 cytoplasm); N_V) numerical density of ultrastructures (number in 1 μ^3 of cytoplasm). Asterisk indicates significant difference from control.

After recovery of the blood flow in the liver atrophic changes were observed at the subcellular level in the rat hepatocytes. For instance, after 2 h of recirculation the number of attached and free ribosomes continued to decrease (Fig. 2, group 3), the glycogen content was reduced by 19% (Table 1), cisterns of the RER remained narrowed, but the surface area of the membranes of RER also was reduced. At this stage the total volume of the lysosomes increased, mainly on account of an increase in the contribution of secondary lysosomes, evidence of activation of the lysosomal apparatus.

The total volume of the mitochondria 2 h after removal of the clamp was 44% greater than in the control, and the area of the outer mitochondrial membrane was increased by 48%, evidence of hyperplasia of the mitochondria.

After 12 h of recirculation the number of mitochondria was the same as in the control, but at the same time the total volume and surface area of the outer mitochondrial membrane remained greater than the control values throughout the period of investigation after restoration of the blood flow. Starting from 24 h of recirculation the area of the inner mitochondrial membrane increased gradually until the 14th day, when it was 52% greater than this parameter in the control. These changes were evidently linked with intensification of the energy-forming function of the mitochondria.

The number of free ribosomes, including ribosomes in polysomes, was restored after 24 h of recirculation, and thereafter continued to rise steadily. After 24 h of recirculation the number of polysomes also was increased (Fig. 2, groups 5-8). Before this stage of recirculation the number of polysomes was the same as in the control.

Hence, after 24 h of recirculation, against the background of structural changes indicating an increased intensity of mitochondrial function, the number of polysomes in the hepatocytes increased, and this can evidently be taken as evidence of activation of synthetic processes in the hepatocytes. In fact, the total surface area of the membrane of the cytoplasmic organelles of the hepatocytes began to increase after 24 h of recirculation (Fig. 3, groups 5-8), and it remained high until the 14th day of recirculation. This is evidence of an increased intensity of synthesis, aimed at meeting the needs of the hepatocytes themselves.

The number of attached ribosomes was reduced throughout the period of recirculation (Fig. 2, groups 3-8). The maximal number of lipid inclusions was observed after 24 h (Table 1), when the total volume of lipid granules was 10 times greater than in the control. The bulk density of the lipid inclusions thereafter decreased gradually, but had not reached the control level on the 14th day of recirculation. Accumulation of lipids and their slow excretion from the hepatocytes were evidently due to disturbance of the function of RER because of re-

duced synthesis of transport proteins, as was shown indirectly by a decrease in the number of attached ribosomes.

Starting from 24 h of recirculation, the dominant processes in the hepatic parenchyma were thus those of restoration of hepatocyte ultrastructure, whereas synthetic processes, aimed at export, were evidently depressed both during ischemia and throughout the period of recirculation until the 14th day. This was shown indirectly by a decrease in the number of attached ribosomes and some delay in lipid transport.

Some workers consider that 30 min of ischemia is a safe period of exclusion of the liver from the circulation in man [1] and in animals [2, 4, 5]. The results of the present investigation indicate that hepatic ischemia for 30 min leads to serious changes in the hepatocytes at the subcellular level, and that complete recovery of the ultrastructure of the hepatic parenchyma has not taken place even by the 14th day of recirculation. This state of affairs raises the question of the need to search for ways of correcting this situation in order to prevent possible complications in the postischemic period or after other states associated with disturbance of the blood flow in the liver.

LITERATURE CITED

1. G. I. Veronskii, Anatomo-Physiological Aspects of Resection of the Liver [in Russian], Novosibirsk (1983).
2. A. Bernelli-Zazzera and G. Gaja, Exp. Mol. Pathol., 3, 351 (1964).
3. I. D. Bowen and R. A. Lockshin, Cell Death in Biology and Pathology, London (1981).
4. K. R. Chien, J. Abrams, R. G.Pfau, et al., Am. J. Pathol., 88, 539 (1977).
5. J. J. Frederiks, K. S. Bosch, M. J. R. Schröder, et al., Exp. Pathol., 22, 245 (1982).
6. E. R. Weibel, Int. Rev. Cytol., 26, 235 (1969).

A POSSIBLE MORPHOLOGIC APPROACH TO THE ASSESSMENT OF MITOCHONDRIAL ENERGY POTENTIAL

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KEY WORDS: mitochondria; energy capacity.

Modern cytopathology has many facilities for obtaining objective morphometric data on the state of structural and chemical components of the cell. Nevertheless, not even this level of cytomolecular research can always provide exhaustive information on the state of function of the cell and, in particular, of its mitochondria. Technical papers dealing with the assessment of energy efficiency of the mitochondria have been published [1]. In the writer's view, for a more complete assessment of the state of mitochondrial function it is preferable to use a conventional parameter (coefficient) which would characterize the potential energy capacity or level of power of the mitochondria, having in mind not the physical definition, but the translated meaning of the latin word "potentia" — meaning power. In the writer's view, analysis of a set of enzyme-histochemical and ultrastructural data relating to the ability of mitochondria to engage in oxidation-reduction reactions with electron transfer along the respiratory chain, could provide an objective idea of such a parameter, for it is these reactions that are components of respiratory assemblages, and directly precede energy production. It is now possible to obtain exact information about components of respiratory assemblages such as electron transfer enzymes — NADH-dehydrogenase (NADH-DH), succinate dehydrogenase (SDH), and cytochrome oxidase, as well as additional enzymes, such as D-β-hydroxybutyrate dehydrogenase and α-glycerophosphate dehydrogenase, which are firmly bound with respiratory fragments. These enzymes of energy production perform vector processes

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