EFFECT OF EXOGENOUS CYTOCHROME c ON OXIDATIVE PHOSPHORYLATION IN THE LIVER OF RATS WITH ACUTE TOXIC HEPATITIS

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In rats with acute toxic hepatitis caused by a single subcutaneous injection of CCl_4, the subcutaneous injection of cytochrome c in a dose of 10 mg/kg stimulates respiration and phosphorylation in the liver and increases the coupling between these processes.

Poisoning with CCl_4 causes damage to the structure and function of the liver, changes the activity of several enzyme systems, disturbs electron transport in the respiratory chain, and uncouples the processes of respiration and phosphorylation [3, 6, 11]. One of the factors reducing the energy potential of the liver under these conditions is the reduced oxygenation of the substrates, associated with damage to the mitochondrial membranes and loss of cytochrome c. It has been shown [2] that the loss of cytochrome c is acute toxic hepatitis is reversible and that the addition of exogenous cytochrome c to the incubation medium restores the rate of substrate oxidation to levels characteristic of the intact liver mitochondria [2].

The fact that the intensity of respiration and phosphorylation is increased on the addition of exogenous cytochrome c to the cytochrome c-deficient liver mitochondria has frequently been observed [5, 8]. It has also been shown that the parenteral administration of cytochrome c to animals with tumors stimulates the disturbed processes of oxidative phosphorylation in the liver mitochondria [5].

The effect of parenteral injection of cytochrome c on oxidative phosphorylation was studied in liver homogenates from rats with acute toxic hepatitis.

EXPERIMENTAL METHOD

Rats weighing 160-220 g were used. The animals were divided into 3 groups. The rats of group 1 received a subcutaneous injection of 40% CCl_4 solution in peach oil in a dose of 0.25 ml/100 g body weight and were sacrificed 1, 3, and 7 days after poisoning. The rats of group 2, starting from the day of poisoning, received daily subcutaneous injections of cytochrome c (Leningrad Meat Combine Medical Preparations Factory, injection grade) in a dose of 10 mg/kg body weight. The control animals (group 3) received equivalent volumes of peach oil and physiological saline instead of CCl_4 and cytochrome c.

After decapitation of the rats the liver was quickly removed, placed in cold 0.25 M sucrose, containing 0.01 M Tris-HCl, pH 7.4, and 0.001 M EDTA, chopped with scissors, and homogenized in a glass homogenizer. The tests were carried out on 1 ml of the 40% homogenate. The intensity of respiration was measured in a Warburg apparatus [12]. The intensity of phosphorylation was estimated from the decrease in the content of inorganic phosphorus in the incubation medium during incubation for 20 min at 37° C. The incubation medium contained (in μmoles) $MgCl_2$ 10, phosphate 40, NaF 30, ADP 2.5, α-ketoglutarate 30. Totalvolume of the sample 2 ml, atmosphere air. The oxygen consumption (ΔO) was ex-

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TABLE 1. Effect of Exogenous Cytochrome c on Oxidative Phosphorylation in Liver Homogenates from Rats with Acute Toxic Hepatitis (M ± m)

Time after poisoning	No. of animals	ΔO (in μ atoms)	%	Δ P (in μ atoms)	%	P/O	%
Control	10	5,51±0,20	100	6,29±0,30	100	1,14±0,06	100
1st day:							
CCl_4	9	3,81±0,17	69	2,01±0,25	32	0,52±0,06	46
CCl_4 + cytochrome c	10	4,00±0,08	72	3,16±0,10	50	0,79±0,02	69
P		>0,05		<0,001		<0,001	
3rd day:							
CCl_4	10	3,82±0,16	69	2,73±0,07	43	0,71±0,03	62
CCl_4 + cytochrome c	10	4,19±0,18	76	3,61±0,10	57	0,86±0,03	75
P		>0,05		<0,001		<0,002	
7th day:							
CCl_4	12	4,37±0,11	79	3,86±0,13	61	0,88±0,03	77
CCl_4 + cytochrome c	9	4,84±0,07	88	5,08±0,09	81	1,05±0,02	92
P		<0,002		<0,001		<0,001	

Legend. Value of P given for group 3 compared with group 2.

pressed in microatoms O_2/10 mg protein per time of incubation, and the decrease in inorganic phosphorus in the medium (ΔP) in microatoms P/10 mg protein per time of incubation. The inorganic phosphorus content was determined by the method of Fiske and Subbarow [7] and protein by Lowry's method [11].

Statistical analysis of the results was carried out by the usual methods [4].

EXPERIMENTAL RESULTS AND DISCUSSION

The experimental results are given in Table 1. They show that injection of CCl_4 into the rats was followed by a decrease in the oxygen assimilation by the liver homogenates and by inhibition of phosphorylation. The most marked changes were found after 1 and 3 days. On the 7th day a tendency was observed for respiration and phosphorylation to return to normal but their intensity was much lower than in the healthy rats and the P/O ratio was only 77% of normal.

Subcutaneous injection of cytochrome c into the rats poisoned with CCl_4 led after the first day to increased esterification of inorganic phosphate, reaching 81% of the phosphorylating activity of the homogenates from the intact animals. The oxygen assimilation increased at the same time, but the differences after 1 and 3 days were not significant. The P/O ratio reached 69% after 1 day, 75% after 3 days, and 92% of normal after 7 days.

Parenteral injection of cytochrome c in acute CCl_4 poisoning thus led to an increase in the intensity of respiration and phosphorylation in the rat liver homogenates and in the degree of coupling of these processes.

The mechanism of normalization of the energy metabolism in the liver of rats with acute CCl_4 poisoning is not yet known, although cytochrome c has been successfully used therapeutically in various fields of medicine.

Cytochrome c is known to be extramitochondrial in origin and, unlike other proteins that are electron carriers, it is synthesized in the endoplasmic reticulum and then built in the ready-made form into the mitochondrial membrane. Meanwhile cytochrome c is highly mobile and readily solubilized [9]. The cytochrome c-deficient liver mitochondria obtained in this way partly or completely lose their ability to carry out coupled oxidative phosphorylation. It has recently been shown that not only damaged, but also intact liver mitochondria are able to take up exogenous cytochrome c [1].

Stimulation of oxidative phosphorylation in the liver tissues of rats with acute CCl_4 poisoning and by parenteral injection of cytochrome c may thus be connected with the penetration of cytochrome c into the cell and its direct action on the intracellular structures of the liver.

LITERATURE CITED

1. M. V. Golubtseva, Ukr. Biokhim. Zh., No. 4, 532 (1971).
2. V. V. Lyakhovich, A. V. Dolgov, V. M. Mishin, et al., Farmakol. i Toksikol., No. 2, 243 (1972).
3 A. Ya. Maiore, M. A. Bolotova, G. Ya. Strozhe, et al., Izv. Akad. Nauk Latv. SSR, No. 7, 92 (1971).
4 N. A. Plokhinskii, Biometrics [in Russian], Moscow (1970).
5. V. I. Firsova, The Effect of Cytochrome c and Catalase on Growth of a Lymphosarcoma and the State of Coupled Oxidative Phosphorylation in the Mitochondria of Animals with Tumors, Author's Abstract of Candidate's Dissertation [in Russian], Leningrad (1969).
6. A. Fischer, Physiology and Experimental Pathology of the Liver, Budapest (1961).
7. C. H. Fiske and J. Subbarow, J. Biol. Chem., 81, 629 (1929).
8. E. E. Jacobs and D. R. Sanadi, J. Biol. Chem., 235, 531 (1960).
9. A. Lehninger, The Mitochondrion, Benjamin.
10. O. H. Lowry et al., J. Biol. Chem., 193, 265 (1951).
11. V. V. Lyachovich (Lyakhovich), V. M. Mishin, A. V. Dolgow (Dolgov), et al., Biochem. Pharmacol., 20, 1437 (1971).
12. W. W. Umbreit, R. H. Burris, and J. F. Staufferd, Manometric Methods for the Study of Tissue Metabolism [in Russian], Moscow (1951).