STUDY OF SOME MITOCHONDRIAL ENZYMES IN CIRCULATING LYMPHOCYTES DURING HEMOPERFUSION FOR EXPERIMENTAL HYPERCHOLESTEROLEMIA

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Analysis of data in the literature on the enzyme cytochemistry of lymphocytes reveals the presence of marked changes in metabolism of the lymphoid system in various pathological states [1, 3, 8, 10]. Enzymes of all the most important energy cycles and pathways can be detected in peripheral blood lymphocytes [9].

The chief source of accumulation of the energy reserves of the cell is the respiratory chain of the mitochondria, which is a system of carriers of the proton and electron of hydrogen from oxidation substrates to oxygen; investigators have accordingly concentrated their attention on dehydrogenases, which catalyze oxidation–reduction reactions. In particular, a definite relationship has been established between the enzymic status of the lymphocytes and the severity of the course of myocardial infarction. Marked changes in dehydrogenase activity of the lymphocytes have been discovered in various forms of clinical and experimental hypoxia [2].

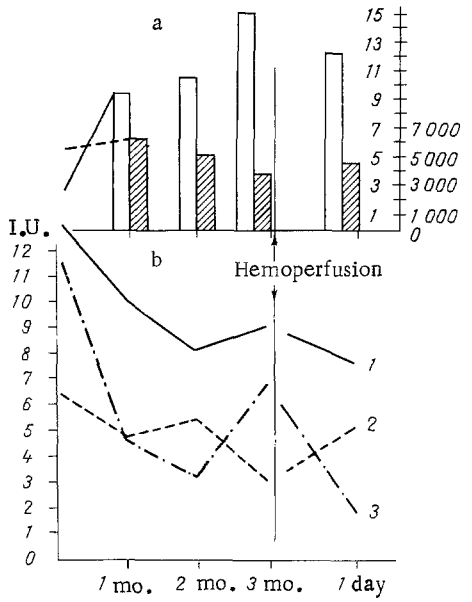

Fig. 1. Time course of changes in dehydrogenase activity during feeding of rabbits with cholesterol, before and after hemoperfusion. a: Shaded columns indicate number of lymphocytes ($\times 10^9$/liter), unshaded columns — serum cholesterol level (in mM); b: 1) SHD, 2) α-GPDH, 3) β-HBDH.

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TABLE 1. Changes in Activity of Some Dehydrogenases (in I.U.) in Circulating Lymphocytes and Serum Cholesterol Concentration (in mM) during Hemoperfusion for Experimental Atherosclerosis (M ± m)

Parameter	Before experiment	Cholesterol feeding for			After hemoperfusion
		1 month	2 months	3 months	
Cholesterol	2,58±0,13	9,14±0,7	11,6±0,7	15,29±1,67*	12,16±1,56
P		<0,001	<0,001	<0,001	>0,1
SDH	13,7±1,0	10,0±1,0	8,1±0,64	9,1±0,72	7,7±1,8
P		<0,02	<0,001	<0,01	<0,05
V	43,06	60,3	24,69	36,4	74,21
A	0,67	0,47	11,34	21,26	0,94
α-GPDH	6,4±0,62	4,8±0,7	5,4±0,7	3,1±0,3	5,1±0,9
P		<0,05	<0,02	<0,001	>0,1
V	47,05	36,0	70,02	48,71	49,8
A	0,56	3,77	0,41	1,68	—0,24
β-HBDH	11,57±2,33	4,72±0,91	3,13±0,67	6,85±1,26	1,88±0,42
P		<0,01	<0,01	<0,05	<0,05
V	49,26	61,0	70,93	71,34	45,21
A	—0,462	2,99	0,72	1,15	0,047

Legend. Data after hemoperfusion compared with data for feeding for 3 months.

There have been few studies of the effect of alimentary hypercholesterolemia and also of decholesterolization by hemoperfusion on the oxidation—reduction enzymes of lymphocytes, and as a rule they have been conducted at the morphological level. The ability of lymphocytes to undergo enzymic adaptation under these conditions has not yet been studied.

The investigation described below was undertaken to discover whether changes occur in the activity of succinate dehydrogenase (SDH), mitochondrial α-glycerophosphate dehydrogenase (α-GDPH), and β-hydroxybutyrate dehydrogenase (β-HBDH) during hemoperfusion for alimentary hypercholesterolemia.

EXPERIMENTAL METHODS

Hypercholesterolemia was induced by daily peroral administration of 1 g cholesterol, added to shredded cabbage fed to male chinchilla rabbits weighing 4-5 kg. The animals were divided into two groups: control and experimental. The experimental rabbits (n = 20) were kept on the atherogenic diet for 3 months. At the end of this time the animals were treated by hemoperfusion. IGI-3 was used as the absorbent. Enzyme activity was determined cytochemically, using p-nitrotetrazolium violet as the hydrogen acceptor [4]. Staining was carried out in accordance with a general scheme, but with different substrates: sodium succinate for SH, sodium β-hydroxybutyrate for β-HBDH, sodium α-glycerophosphate for demonstration of α-GPDH. Control films were incubated in the absence of substrate from the incubation medium. Tetrazolium salts, which are acceptors of reduced equivalents, on reacting with the hydrogen substrates form stained granules of formazan which are insoluble in water. The number of granules of reaction products was counted [7]. The technique of quantitative cytochemical analysis developed by Nartsissov [6] was used.

EXPERIMENTAL RESULTS

The results are evidence that during feeding of rabbits with cholesterol its level in the serum rises sharply depending on the duration of feeding (Table 1). In the first stages of cholesterol administration the activity of the dehydrogenases chosen for study was found to be reduced, and the absolute number of lymphocytes fluctuated, with a marked decrease in their number after 3 and 5 months ($P < 0.05$; Fig. 1).

In rabbits of the control group the level of α-GPDH activity at all times of observation was lower than that of SDH activity. In the experimental rabbits after cholesterol feeding for 3 months SDH activity was reduced by 34% ($P < 0.01$) and α-GPDH activity was reduced by 50% ($P < 0.001$, Table 1), evidence of reduced activity of the glycerophosphate shunt, which maintains normal cell respiration. Values of coefficients of variation (V) and asymmetry (A) varied considerably for SDH and α-GPDH in individual lymphocytes during feeding, evidence of the formation of a separate pool of cells with reduced activity of the glycerophosphate shunt and Krebs' cycle.

It will be clear from Table 1 that β-HBDH activity was reduced by 73% ($P < 0.01$) by the end of the second month of feeding. During continued cholesterol feeding activation of β-HBDH was observed. After feeding for 3 months both SDH and β-HBDH showed a tendency toward activation. This can be explained by intracellular metabolic adaptation to cholesterol administration (to cholesterol poisoning).

The lymphocytes differ significantly in activity of the investigated enzymes. Cells with large quantities of granules and cells which do not exhibit enzyme activity are observed; i.e., targeted cells, cellular substances with the corresponding enzymic status are isolated; apparently, on the levels of which cholesterol is realized as an extremal factor. This heterogeneity of cells is reflected in the coefficient of variation, characterized by the degree of variation in percentages. As seen in Table 1, this coefficient is very significant but in this case 1-2% of the cells are of separate pools, probably evidence of different populations. Statistical parameters of higher orders indicate that the distribution of lymphocytes with respect to enzymic activity is asymmetric. The coefficient of asymmetry A is greater than zero in two cases, i.e., the population variants with values exceeding the arithmetic mean predominate. A statistically reliable correlation between degree of activity of the various enzymes and characteristics of the cell distribution in common populations exists.

The results are thus evidence of the existence of definite coupling between metabolic processes in the lymphocyte population of rabbits with experimental atherosclerosis.

We selected the technique of repeated hemoperfusion, which is an optimal method of prevention and treatment of atherosclerosis [5]. The results of the investigation 1 day after a single hemoperfusion procedure showed that activity of SDH and β-HBDH had fallen lower still, but α-GPDH activity showed a tendency to recover (Table 1), evidence of normalization of operation of the glycerophosphate shunt and, consequently, an improvement of cell respiration.

The study of oxidative metabolism under conditions of alimentary hypercholesterolemia thus revealed profound disturbances in the energy-producing system of the cell, i.e., a decrease in the basic parameters of oxidative metabolism reflects a disturbance of the energy function of the mitochondria. Alimentary hypercholesterolemia from the very beginning of the experiment led to a disturbance of balance between processes of anabolism and catabolism, reflecting the state of oxidative processes in the Krebs' cycle.

It can be tentatively suggested that such a combination of changes in the dehydrogenase system during hypercholesterolemia reflects the initial periods of atherosclerosis. After removal of cholesterol from the body by a single hemoperfusion some tendency was observed for the circulating lymophocytes to be brought out of their state of hypoxia. The facts described above are also evidence that the study of dehydrogenase activity, from the writers' point of view, must be used for the diagnosis and evaluation of the treatment of atherosclerosis under experimental and clinical conditions.

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