OXIDATION – REDUCTION PROCESSES DURING ALLERGIC REACTIONS OF THE DELAYED TYPE TO MICROBIAL ANTIGENS

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The effect of the general allergic reaction of delayed type (GARDT) to brucellosis antigen on glycolytic processes and activity of dehydrogenases of the Krebs' cycle was studied in the blood and organs of guinea pigs. Besides inhibition of the activity of the four dehydrogenases studied, inhibition of glycolysis connected with a decrease in the concentrations of lactate and pyruvate and inhibition of lactate dehydrogenase (LD) activity was observed. An increase in the content of anaerobic fractions and a decrease in the content of aerobic fractions were observed in the LD isozyme spectrum, together with excessiveness of the spectrum itself.
KEY WORDS: allergic reaction of delayed type; microbial antigen; glycolysis; dehydrogenase activity; lactate dehydrogenase and its isozymes.

As the writers showed previously [6] allergic reactions of immediate type were accompanied by profound changes in oxidation–reduction processes accompanied by marked inhibition of dehydrogenation, a disturbance of the function of the cytochrome region of the biological oxidation chain, and a compensatory intensification of glycolysis, leading to uncompensated metabolic acidosis.

The relations between immediate and delayed allergy are complex, but these two types of allergy are closely interconnected and constitute "a closed chain of transitions between anaphylactic reactions of immediate and reactions of delayed type" [1].

The object of the present investigation was accordingly to study the biochemical changes arising during the development of a general allergic reaction of delayed type (GARDT), more especially because no data on this subject can be found in the literature.

EXPERIMENTAL METHOD

Two series of experiments were carried out on 142 guinea pigs: I) control, II) animals with a GARDT to Brucella antigen. The animals were sensitized by a single injection of a living culture of Brucella abortus in a dose of 10^9 bacterial cells. The degree of sensitization was tested 45-50 days after injection of the sensitizing dose by Burnet's test, and was 1.71 ± 0.17 cm^2.

The reacting dose of therapeutic brucellosis vaccine (TBV, manufactured by the Tbilisi Research Institute of Vaccines and Sera, batch 411, No. OBK 1505) was injected intravenously 5-7 days after Burnet's test in a volume of 1 ml (5×10^8 bacterial cells). The intensity of GARDT was judged by the temperature reaction [2, 18]. The animals were investigated at the height of development of the reaction, namely 8 h after injection of the reacting dose of antigen, when the temperature reached a maximum [3], with a mean rise of 1.1°C.

The concentrations of lactic and pyruvic acids [5, 8], and of glucose and glycogen [4, 9], the total activity of lactate dehydrogenase (LD) [16] and its isozymes [10, 11, 15], and activity of three NAD-dependent dehydrogenases of the Krebs' cycle – isocitrate, α-ketoglutarate, and malate dehydrogenases (MD) and of the flavoprotein enzyme succinate dehydrogenase (SD) [13, 14], were determined in the blood and tissues of the experimental animals.

The results were subjected to statistical analysis by means of Student's t-test. Coefficients of paired and multiple correlation were calculated, and, in the case of the LD isozyme spectrum, its entropy and excessiveness.

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TABLE 1. Concentrations of Lactate and Pyruvate and LD Activity (M ± m) in Organs and Blood of Control Guinea Pigs (I) and during GARDT to Brucella Antigen (II)

Index	Series of experi-	Blood	Brain	Heart	Lungs	Liver	Spleen	Kidneys
Lactate, mg/g or mg/100 ml blood	I	8,7±0,7	6,4±1,0	6,3±0,6	5,5±0,7	5,9±0,7	5,9±0,7	5,2±1,8
	II	5,8±0,6*	4,5±0,5*	4,6±0,5*	4,5±0,6	3,2±0,4*	3,7±0,4*	3,6±0,5
Pyruvate, μg/g tissue or mg/100 ml blood	I	0,6±0,1	23,7±3,9	27,2±3,9	44,2±5,2	15,7±2,6	24,9±4,0	19,5±2,6
	II	0,8±0,1	15,9±1,2	15,5±1,4*	23,0±4,0*	21,9±2,0	25,4±2,1	19,8±1,6
Lactate/pyruvate, % of control	II	50	104	128	151	37	62	69
Redox potential, mV	I	—168,7	—129,5	—131,6	—139,8	—125,8	—131,3	—125,2
	II	—178,9	—128,8	—127,9	—133,7	—138,0	—137,6	—134,6
Excess of lactate, mg/g	II	—1,27	0,23	1,09	1,63	—0,60	—1,00	—1,31
LD, μmoles pyruvate/g tissue/h	I	23,6±1,6	568±71	1026±96	365±56	400±70	—	839±81
	II	24,0±7,7	447±52	978±82	249±47	164±3,6*	—	576±75*

* P < 0.05 compared with control.

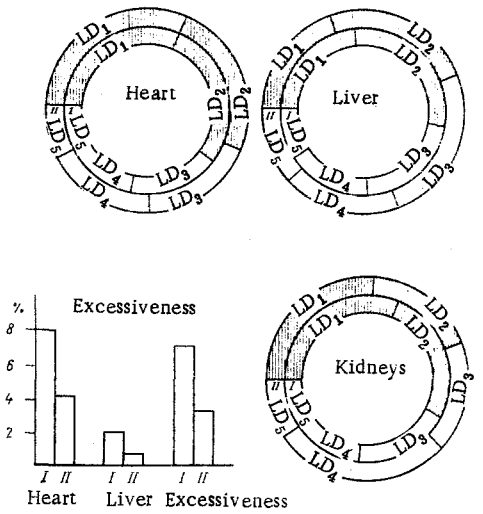

Fig. 1. Distribution of LD isozymes and excessiveness of isozyme spectrum in organs of control guinea pigs (I) and guinea pigs with a GARDT (II).

EXPERIMENTAL RESULTS

During the GARDT to Brucella antigen the lactic acid level was lowered in all organs (Table 1), but most significantly in the liver (by 48%) and muscles (by 89%). The pyruvate concentration fell in the lungs (by 48%), the heart (by 43%), the brain (by 37%), and the muscles.

The lactate/pyruvate ratio fell in most organs and the lactate excess in several organs was negative in value.

The LD activity was significantly depressed in the liver (by 59%), kidneys (by 32%), and muscles (by 16%).

The LD isozyme spectrum showed a decrease in the relative proportion of aerobic fractions and an increase in the anaerobic fractions. The entropy of the spectrum was increased, but its excessiveness was reduced, pointing to instability of this system (Fig. 1).

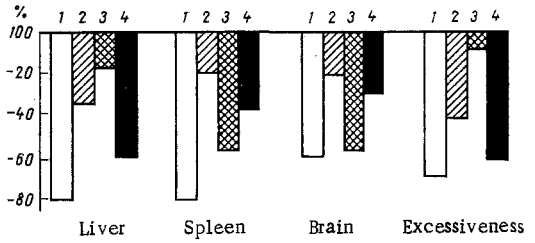

Fig. 2. Activity of dehydrogenases of Krebs' cycle in organs of guinea pigs with GARDT (in % of control). 1) Isocitrate dehydrogenase: 2) α-ketoglutarate dehydrogenase; 3) SD; 4) MD.

Consequently, judging from the data described above, the velocity of glycolysis was reduced, and this led to a decrease in the production of lactic and pyruvic acids. The accumulation of pyruvate in the liver could be the result of considerable inhibition of the subsequent pathways of its metabolism in the Krebs' cycle, for the reaction of dehydrogenation processes to the GARDT to Brucella antigen consisted of a decrease in the activity of all four dehydrogenases studied (Fig. 2).

Isocitrate dehydrogenase, whose activity fell significantly in all organs without exception, reacted most strongly of all. The fact that inhibition of succinic acid was significantly less in those organs in which MD activity fell sharply will be noted (Fig. 2). This was perhaps due to the removal of oxalate inhibition.

The cause of this sharp inhibition of dehydrogenation processes in Krebs' cycle could be a change in the permeability of the membranes with disturbance of the structure of the mitochondria [12]. Fatty acids accumulating in allergic reactionsbecause of increased glycolysis [17] could also have an inhibitory effect on the dehydrogenases. During analysis of coefficients of paired and multiple correlation in the latter case very close direct correlation between the parameters studied was shown, the coefficient being close to unity.

Negative correlation between dehydrogenase activity and the indices of glycolysis and direct correlation between individual dehydrogenases were found in the organs.

Changes discovered during the GARDT to Brucella antigen thus differed qualitatively from changes found by the writers during sensitization by Brucellas [7] and were unfavorable in character, for besides inhibition of dehydrogenases, glycolysis also was depressed. In the presence of a marked blocking of dehydrogenation and a reduction in catalase activity and the concentrations of cytochrome c and myoglobin [7], the writers are inclined to regard this as an extremely unfavorable sign of exhaustion of the compensatory powers of the body, leading to a critical deficiency of the energy required for synthesis of the high-energy compounds that maintain the supply of energy for all the functions of the body.

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