OXIDATION–REDUCTION PROCESSES DURING SENSITIZATION AND THE ALLERGIC REACTION TO MICROBIAL ANTIGENS

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The effect of sensitization and an allergic reaction of delayed type (ARDT) to brucellosis antigen on the state of glycolysis and activity of the dehydrogenases of the Krebs' cycle was studied. Sensitization to brucellas was shown to be accompanied by inhibition of dehydrogenase activity and activation of glycolysis. The changes were more marked in the case of sensitization with living brucellas. In ARDT, besides depression of dehydrogenase activity, glycolysis also was inhibited. In the lactate dehydrogenase isozyme spectrum the content of aerobic fractions and of excess lactate fell both in sensitized animals and in animals with ARDT. Correction of the biochemical disturbances detected in ARDT led to a decrease in the degree of sensitization and to abolition of the ARDT symptom-complex.

KEY WORDS: dehydrogenases; lactate dehydrogenase; lactate dehydrogenase isozymes.

Infectious-allergic states play a leading role among the various forms of allergy because of the high relative contribution of microorganisms to the etiology of allergic diseases and because of the widespread use of microbial antigens for prophylaxis and treatment [1, 2].

Despite this fact, the process of sensitization and development of allergic reactions to various microbial antigens has been inadequately studied: Functional changes in organs and systems have not been explained and the biochemical disturbances developing in association with them have not been discovered.

In the investigation described below the characteristics of oxidation-reduction processes were studied during sensitization and an allergic reaction to microbial antigens.

EXPERIMENTAL METHOD

Experiments were carried out on 208 guinea pigs: Group 1 consisted of control animals; group 2 of animals sensitized by a single subcutaneous injection of 1 billion *Brucella abortus* cells; group 3 of animals sensitized by three subcutaneous injections each of 2 billion *Br. abortus* cells, killed by heat; group 4 of animals with an allergic reaction of delayed type (ARDT) to brucellosis antigen; group 5 of animals with ARDT to brucellosis antigen after nonspecific treatment. The degree of sensitization of the animals was tested 45 days later by Burnet's allergic skin test, the results of which were 1.03 ± 0.7 for group 2, 0.54 ± 0.11 for group 3, 1.7 ± 0.17 for group 4, and 1.82 ± 0.31 cm^2 for group 5, respectively. After Burnet's test the animals of group 5 received 10 mg ascorbic acid, 2 mg riboflavin, 10 mg methionine, 250 mg glucose, 0.05 ml of a 5% solution of pyridoxin, and 0.1 ml of a 1% solution of sodium adenosinetriphosphate daily for 10 days. The concentrations of lactic and pyruvic acids [4, 5], glucose, and glycogen [3, 7], and activity of lactate dehydrogenase (LD) and its isozymes [6, 8, 9, 12, 13] and the dehydrogenases of the Krebs' cycle [10, 11] were determined in the blood and tissues of the animals 45–60 days after sensitization and 8 h after intravenous injection of a reacting dose of therapeutic brucellosis vaccine (500 million bacterial cells).

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TABLE 1. Concentrations of Lactate, Pyruvate, and Glycogen and LD Activity in Control Guinea Pigs (I), in Animals Sensitized with Living (II) and Killed (III) Culture of *Br. abortus*, and in Animals with ARDT to Brucellosis Antigen (IV), M ± m

Index		Blood	Brain	Heart	Lungs	Liver	Spleen	Kidneys
Lactate, mg/g tissue 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	17,8±1,1*	16,2±1,4*	13,1±1,6*	18,5±1,4*	12,8±1,3*	14,9±2,0*	16,9±2,0*
	III	12,9±0,8*	13,6±1,0*	13,1±0,5*	14,8±0,4*	10,7±0,2*	12,8±0,3*	12,6±0,6*
	IV	5,9±0,6*	4,5±0,5*	4,6±0,6*	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	0,62±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	0,6±0,1	12,7±1,6*	13,1±3,2*	31,1±1,8*	6,3±0,9	14,6±1,1*	23,8±2,3
	IV	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 ratio (% of control)	II	202	473	432	483	557	430	267
	IV	50	104	128	151	37	62	69
Redox potential, mV	I	—168,7	—129,4	—131,6	—139,8	—125,8	—131,3	125,2
	II	—159,5	—109,5	—111,5	—119,0	—102,4	—111,6	—116,5
	IV	—178,9	—128,8	—127,9	—133,7	—138,0	—137,6	—134,6
Lactate excess, mg/g	II	9,02	12,79	10,08	14,68	10,61	11,39	10,41
	IV	—1,27	0,23	1,09	1,63	—0,6	—1,0	—1,31
Glycogen, mg/g tissue, or blood glucose, mg/100 ml	I	140,1±11,5	26,0±4,5	32,5±6,0	25,4±3,4	110,4±29,2	26,9±2,8	12,3±1,7
	II	246,0±13,1*	39,5±5,3	36,9±6,2	25,7±4,1	68,2±20,5	30,4±3,4	20,2±3,0
	IV	145,8±21,4	24,7±7,6	30,4±6,3	22,4±8,8	30,9±6,8	17.5±7,0	17,2±8,5
LD, μmoles pyruvate/g tissue/h	I	23,6±1,6	568±71	1025±96	365±56	400±70	—	839±81
	II	25,0±3,9	671±52	766±27	346±29	370±31	—	651±79
	III	12,2±0,8*	514±37	528±76*	483±69	352±11	—	452±32
	IV	24,0±7,7	447±52	978±82	249±47	164±3,6*	—	576±75*

*Results differing significantly from control.

The results were calculated per gram tissue dried to constant weight and subjected to statistical analysis by Student's t-test. Coefficients of correlation were calculated. The entropy and excess were calculated for LD isozymes.

EXPERIMENTAL RESULTS

Sensitization of the guinea pigs with brucellas caused a significant increase in the lactate level in the organs and blood, accompanied by an increase in the lactate:pyrivate ratio, a fall of redox potential of the system, and the appearance of an excess of lactate (Table 1). In ARDT a fall in the lactic acid level was observed in all organs and a fall in the pyruvic acid level in the lungs, heart, brain, and muscles. The lactate:pyruvate ratio fell in most organs. No excess of lactate was observed.

Total LD activity was unchanged during sensitization but reduced in the liver, kidneys, and muscles of the animals with ARDT. Besides a reduction in the aerobic fractions, a decrease in excess also took place in the LD isozyme spectrum of the sensitized animals and animals with ARDT (Fig. 1).

Inhibition of dehydrogenation of the substrates of the Krebs' cycle observed in all organs of animals sensitized with living brucellas deepened during the development of the allergic reaction, and in animals with ARDT the strongest reaction was given by isocitrate dehydrogenase, whose activity fell significantly in all organs without exception (Fig. 2). The much lesser degree of inhibition of succinate dehydrogenase in organs in which malate dehydrogenase activity was sharply reduced will be noted. This was probably due to the removal of oxalate inhibition. Negative correlation was found between dehydrogenase activity and the indices of glycolysis and positive correlation between activity of the individual dehydrogenases (Fig. 3). In the writer's view changes discovered in dehydrogenation of the substrates of the Krebs' cycle constitute the main component of the disturbances of oxidation-reduction processes in allergy to brucellosis antigen, leading to a decrease in ATP production and determining the switch of energy metabolism of the body from the oxidative to the glycolytic

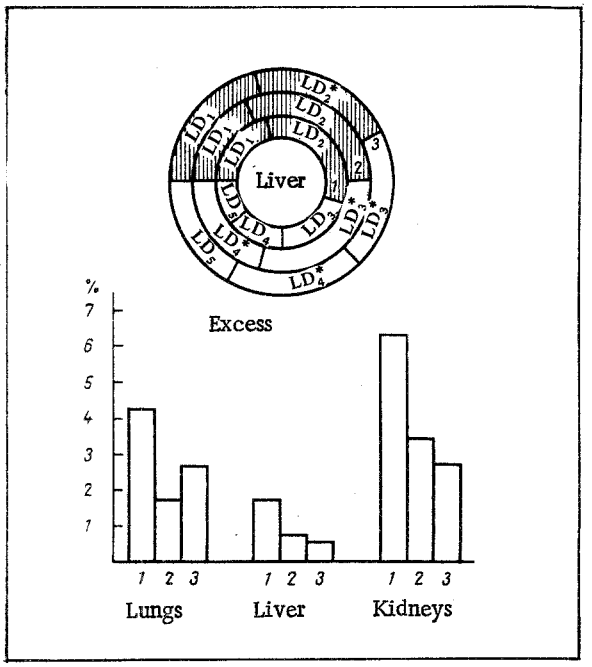

Fig. 1

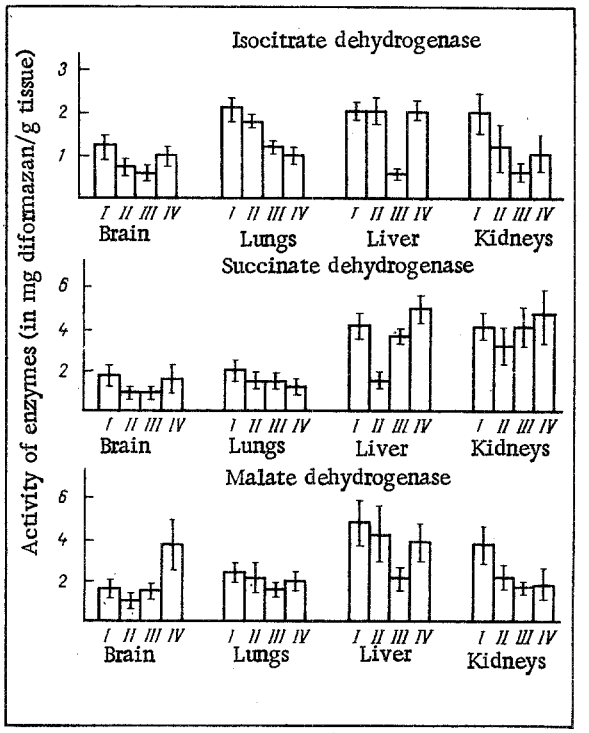

Fig. 2

Fig. 1. Distribution of LD isozymes (in % of total activity) and excess of isozyme spectrum in control guinea pigs (1), animals sensitized with living brucellas (2), and animals with allergic reaction of delayed type (3). Data differing significantly from control marked by asterisk.

Fig. 2. Activity of dehydrogenases of Krebs' cycle in control guinea pigs (I), animals sensitized with living brucellas (II), and animals with allergic reaction before (III) and after treatment (IV).

pathway. The reason for inhibition of the dehydrogenases was probably a change in membrane permeability and structural disturbances in the mitochondria [14].

The comparison showed that vaccination of animals with living and killed cultures of brucellas caused basically identical changes in oxidation—reduction reactions. Presumably the state of increased sensitivity was the most important factor in the disturbances discovered, although the infectious process arising after injection of living vaccine strains must also have influenced the various indices: This effect was manifested as the sharper changes after injection of the living brucellas. However, this result could also have been due to differences in the degree of sensitization and to repeated antigenic action arising during administration of the killed brucellas, and differences in the dose of antigen given.

To sum up the changes discovered during the ARDT to brucellosis antigen, it will be clear that they differed qualitatively from the changes observed in animals sensitized with brucellas and were unfavorable in character, for besides inhibition of dehydrogenases, glycolysis also was inhibited. In the presence of a marked block to dehydrogenation, this must be regarded as an unfavorable sign of exhaustion of the compensatory powers of the animal, with a resulting deficiency of energy required for the synthesis of high-energy compounds.

Consequently, the animals of group 5, at the height of development of sensitization to brucellas, were given a 10-day course of treatment aimed at maintaining the energy reserves of the body. As a result of treatment the readings of Burnet's allergic skin test were reduced by 3.5 times compared with the initial value to 0.52 ± 0.15 cm^2. The clinical symptom-complex of ARDT following injection of the reacting dose of antigen did not develop and the body temperature was unchanged. Moderate stimulation of glycolysis was observed in the blood and organs, with an increase in lactate production, activation of LD, and hyperglycemia. These changes were accompanied by abolition of the dehydrogenation block in the Krebs' cycle (Fig. 2) and were evidence of beneficial changes in energy metabolism.

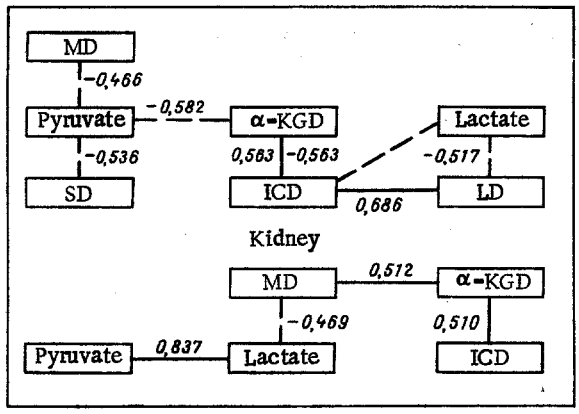

Fig. 3. Diagram showing correlations between parameters studied during allergic reaction of delayed type. Numbers represent values of coefficients of correlation. Continuous line — positive correlation; broken line — negative correlation.*

The experiments thus showed that, in principle, the degree of sensitization and the severity of the ARDT to microbial antigens can be controlled in principle under experimental conditions by correction of biochemical disturbances without the use of immunodepressants and antimediator agents.

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*MD, malate dehydrogenase; SD, succinate dehydrogenase; α-KGD, α-ketoglutarate dehydrogenase; ICD, isocitrate dehydrogenase; LD, lactate dehydrogenase.