5. D. E. Gregg, E. M. Khoure, D. E. Donald, et al., Circulat. Res., **31**, No. 2, 129 (1972).
6. M. A. Heymann, B. D. Payne, J. I. E. Hoffman, and A. M. Rudolf, Cardiovasc. Dis., **20**, No. 1, 55 (1977).
7. K. G. Lurie, M. E. Billingham, S. W. Jamieson, et al., Transplantation, **31**, No. 1, 41 (1981).
8. H. E. Morgan, J. R. Neely, and Y. Kira, Basic Res. Cardiol., **79**, No. 3, 292 (1984).

ACTIVITY OF SOME ENZYMES OF CARBOHYDRATE METABOLISM IN THE LYMPH DURING A FEBRILE REACTION

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Much research has been devoted to the study of changes in enzyme activity in the tissues and peripheral blood during the febrile reaction (FR). However, the character and direction of changes in individual enzymes in the lymph have not yet been reflected in the literature. It can be tentatively suggested that disturbances of enzyme activity of individual organs and tissues in pathological processes will be exhibited in the outflowing lymph earlier than in the blood, for enzymes, being high-molecular-weight compounds, can pass from intercellular connective-tissue spaces at their site of release into the general circulation only after they have undergone resorption from lymphatic capillaries.

For this reason we undertook a comparative study of activity of aldolase and lactate dehydrogenase (LDH) and its isozymes in the lymph and blood during the course of FR of varied duration.

EXPERIMENTAL METHOD

Experiments were carried out on 63 chinchilla rabbits weighing from 2.5 to 4.2 kg. FR was produced by intravenous injection of pyrogenal by the method described previously [7]. Animals receiving injections of pyrogen-free physiological saline, made up in bidistilled water, were used as the control. Lymph was obtained from the thoracic lymph duct (TLD) at the point where it empties into the venous angle, and blood for investigation was taken from the femoral vein. Activity of aldolase [2, 11] and LDH [9, 13] and its isozymes [8] was determined in the lymph and blood at intervals during FR of varied duration. The experimental results were subjected to statistical analysis. After the experiment the animals were killed by injection of a lethal dose of general anesthetic.

EXPERIMENTAL RESULTS

It will be clear from Tables 1 and 2 that total LDH activity in lymph from TLD was 1.6 times higher, and aldolase activity 1.5 times lower than in the blood serum.

Irrespective of the duration of FR, it was accompanied by marked activation of the enzymes tested in the body fluids. For instance, in the stage of elevation of the body temperature (2.5-3 h after the beginning of pyrogenal injection) an increase was observed in LDH activity in both lymph and blood. Activity of all the isozymes also increased, although this was more marked relatively in the case of LDH_4 and LDH_5 (by 3-4 times). In the stage of falling temperature, LDH activity in the biological fluids continued to remain high.

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TABLE 1. Activity of Aldolase, LDH, and Its Isozymes (in μmoles/liter·sec) in Thoracic Duct Lymph of Rabbits during Febrile Reaction ($M \pm m$, $n = 7$)

Name of enzymes	Control animals	Injection of pyrogenal							
		single	3 times			5 times			10 times
		after 2½, 3 h	after 5-5½ h	4th day	6th day	10th day	6th day	10th day	11th day
Aldolase	0,11±0,01	0,37±0,05*	0,40±0,10**	0,46±0,07*	0,40±0,06*	0,13±0,03	0,51±0,06*	0,39±0,07*	0,63±0,07*
LDH total	1,05±0,08	1,66±0,27*	1,66±0,11*	1,99±0,09*	1,52±0,10*	0,87±0,13	2,39±0,07*	2,04±0,14*	2,40±0,05*
LDH_1	0,32±0,03	0,48±0,03*	0,43±0,03*	0,50±0,03*	0,49±0,04*	0,28±0,04	0,56±0,02*	0,60±0,05*	0,62±0,02*
$LDH_{2,3}$	0,66±0,05	1,01±0,07*	1,00±0,06*	0,69±0,07*	0,94±0,06*	0,55±0,08	1,55±0,07*	1,26±0,09*	1,48±0,04*
$LDH_{4,5}$	0,07±0,01	0,17±0,01*	0,23±0,03*	0,23±0,02*	0,10±0,02	0,04±0,01*	0,28±0,01*	0,18±0,03*	0,30±0,01*

Legend. Here and in Table 2, $*p < 0.05$.

TABLE 2. Activity of Aldolase, LDH, and its Isozymes (in μmoles/liter·sec) in Blood Serum from Femoral Vein of Rabbits during Febrile Reaction ($M \pm m$, $n = 7$)

Name of enzymes	Control animals	Single after 2½-3 h	Injection of pyrogenal						
			3 times			5 times			10 times
			after 5-5½ h	4th day	6th day	10th day	6th day	10th day	11th day
Aldolase	0,18 ±0,04	0,59* ±0,05	0,29 0,04	0,38* 0,04	0,34* 0,03	0,30* 0,03	0,58* 0,04	0,37* 0,03	0,68* 0,05
LDH total	0,66 ±0,07	1,47* ±0,12	1,80* 0,14	2,29* 0,11	0,68 0,06	0,78 0,07	2,42* 0,10	1,15* 0,10	2,55* 0,14
LDH_1	0,18 ±0,02	0,39* ±0,04	0,46* 0,04	0,58* 0,05	0,22 0,02	0,26* 0,02	0,54* 0,03	0,34* 0,05	0,63* 0,06
$LDH_{2,3}$	0,44 ±0,05	0,95* ±0,09	1,16* 0,09	1,46* 0,10	0,42 0,04	0,49 0,04	1,58* 0,07	0,75* 0,06	1,59* 0,06
$LDH_{4,5}$	0,03 ±0,01	0,13* ±0,01	0,18* 0,02	0,25* 0,02	0,05 0,01	0,04* 0,01	0,30* 0,02	0,06* 0,01	0,33* 0,03

After three injections of the preparation the LDH level in the lymph increased for a longer time than in the blood, and it was not until the 10th day after the development of fever that its concentration reached its initial values. The increase in total LDH activity in the lymph toward the 6th day of the experiment, moreover, took place on account of LDH_1, LDH_2, and LDH_3.

By contrast with the dynamics of LDH, the rise of the aldolase level in the lymph following one and three injections of pyrogen exceeded 3-4 times.

The trend and duration of the changes in the spectrum of enzymes of carbohydrate metabolism in the body fluids during prolonged (5 and 10 days) FR were the same as after three injections of pyrogenal. The concentrations of LDH isozymes of anaerobic glycolysis (LDH_4 and LDH_5) in the lymph were 4 times higher, and in the blood 10-11 times higher.

Since many intracellular and extracellular factors can influence the activity, rate of synthesis, and degradation of enzymes, a definite role in the explanation of changes in the LDH and aldolase levels in the lymph and blood during FR can be ascribed to the influence of glucocorticoids on glycolysis in the liver cells and their catabolic effect on muscle tissue [10, 14]. The above remarks are in agreement with the view that FR is accompanied by activation of the hypothalamo-hypophyseoadrenal system [1, 5] and, on the other hand, that injection of hydrocortisone into animals leads to an increase in aldolase and transaminase activity in TLD lymph [6]. The increase in LDH and aldolase activity in the body fluids during FR, together with the supply of these enzymes from liver and muscle tissue, may probably also be connected with their release from the myocardium. Proof of this is given by investigations in which, after injection of bacterial pyrogens, increased LDH activity was found in the liver and myocardium of guinea pigs [12]. At the same time, we know that LDH_1 and LDH_2 are found mainly in the myocardium, LDH_4 and LDH_5 mainly in the liver and skeletal muscles, whereas aldolase is found in all the tissues listed above [3, 4, 14]. An increase in activity of cytoplasmic enzymes in the lymph and blood during FR is connected not only with labilization of cell membranes, but also evidently with the reduced functional ability of the liver to degrade an excess of the enzyme.

We know that in tissues with well developed aerobic metabolism the isozymes LDH_1 and LDH_2 predominate, whereas the glycolytic path of energy formation predominates, LDH_4 and LDH_5 are activated. Accordingly, a steady rise in LDH_4 and LDH_5 activity in the biological fluids in the present investigation can be attributed to increased permeability of cell membranes, intensification of anaerobic processes, and the arrival of the enzymes from the liver and muscle tissue. On the other hand, when changes in the activity of these isozymes are explained, the inhibitory effect of glucocorticoids must also be borne in mind: these

substances depress LDH_4 and LDH_5 as constituents of total LDH activity, and thereby "moderate" the even greater rise of their in the body fluids.

On the whole these investigations lead to the conclusion that enzymes released from the tissues during FR are initially resorbed by lymphatic capillaries, and their levels of activity in the blood serum are largely determined by the transport function of the lymphatic system.

LITERATURE CITED

1. G. Z. Abullin et al., Byull. Éksp. Biol. Med., **89**, No. 6, 669 (1980).
2. V. A. Anan'eva and V. R. Obukhova, in: Biochemical Methods of Investigation [in Russian], Moscow (1965), pp. 24-26.
3. K. A. Andriutsa, Isozymes in Patients with Virus Hepatitis [in Russian], Kishinev (1978), 194 pp.
4. D. Wilkinson, Principles and Methods of Diagnostic Enzymology [Russian translation], Moscow (1981).
5. V. D. Melekhin, Éndokrinologiya (Kiev), No. 13, 40 (1983).
6. M. M. Minnebaev, Prob. Éndokrinol., No. 5, 62 (1970).
7. M. M. Minnebaev and F. I. Mukhutdinova, Byull. Éksp. Biol. Med., **105**, No. 3, 284 (1988).
8. A. Zarembskii (ed.), Determination of Isozymes in Biological Fluids: a Teaching Aid [in Russian], Leningrad (1984).
9. M. D. Podil'chak, Clinical Enzymology [in Russian], Kiev (1967).
10. M. I. Smirnov et al., Vopr. Med. Khim., No. 1, 68 (1968).
11. V. I. Tovarnitsskii and E. N. Voluiskaya, Lab. Delo, No. 6, 7 (1955).
12. L. Tsolova and G. Ugzunova, Ukr. Biokhim. Zh., **47**, No. 4, 465 (1975).
13. M. Sevela and J. Tovarek, Cas. Lek. Cesk., **98**, No. 27, 844 (1959).
14. J. Weissinger et al., Enzyme, **26**, No. 5, 342 (1980).