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The Effect of Muscle Activity on the Lymphatic and Venous Transport of Lactate Dehydrogenase

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Summary

Muscle activity (electrical stimulation of the muscles of the posterior extremity) in dogs with thoracic duct fistula did lead to a fivefold increase of the LDH-activity in leg lymph. A significant increase of enzyme activity was observed also in blood serum. It is concluded, that the enzyme protein is transported from the tissues during muscle activity not exclusively by the lymphatics but also by the veins.

The view, that protein molecules are transported from tissues to the blood stream exclusively by the lymphatic vessels is more or less generally accepted (1, 4). It was however shown that colloides injected into some tissues are to a considerable extent absorbed also by the blood capillares (2, 8). The objection can nevertheless be raised, that in these experiments the colloidal substances were actually driven by the injection pressure through the damaged capillary wall into the vessel lumen.

The activity in blood plasma of the cytoplasmic enzyme lactate dehydrogenase is markedly increased in various pathologic conditions. In these states the enzyme enters into the circulation in consequence of cellular damage or increased membrane permeability. Plasma LDH level is however raised also by hypoxia and muscular activity (3, 6, 7, 11). There is no evidence for the damaging effect of muscular exercise to the capillary wall. Accordingly, LDH leaking during muscle activity into the intercellular fluid may be a suitable molecule for the study of protein transport from the tissues. LDH has the further advantage that its source can be traced back to the tissue of origin by separating electrophoreticaly the component isoenzymes. As it is well established, the individual tissues show a specific isoenzyme pattern and in pathologic conditions the enzyme from the damaged tissues does not only increase LDH activity in plasma but also procedures a characteristic shift in the LDH-isoenzyme pattern (5, 13, 14, 16).

In the experiments to be reported the effect of muscular activity on lymphatic and plasma enzyme activity and LDH-isoenzyme pattern was studied in dogs. Consequently it could be shown that the enzyme protein is transported from the activity tissues by lymphatic as well as by venous channels.

Material and Methods

In 13 mongrel dogs in pentobarbitone anaesthesia a femoral lymph vessel was dissected free and cannulated in the inguinal region distally from the lymph node. The remaining afferent lymphatic channels of the lymph node were ligated. The thoracic duct was dissected in the neck and a plastic tube was introduced into it. Electrodes were

placed on the m.m. biceps femoris, semitendinosus and gracilis and the muscles were stimulated with a square wave impulse generator (50 volt, 12 msec, 8 imp./sec) in two periods, each of 30 minutes duration. Femoral and thoracic duct lymph, arterial and femoral venous blood were collected before the stimulation, after the first and the second period of muscle activity and 1 and 3 hours after the end of the stimulation.

Total LDH activity in lymph and blood serum was estimated according to *Wroblevsky* and *La Due* (15) and expressed as μMol transformed substrate per minute, per ml serum or lymph at 25 °C.

The isoenzymes were separated electrophoretically (12) in 1% agarose gel medium and sodium barbiturate-HCl buffer (pH 8,6, ionic strenght 0,05) on microscopic slides at 7 volt/cm. The slides were developed according to *Van der Helm* (10) and the percentage of the isoenzyme fractions was determined with a Zeiss densitometer.

From the distribution of the isoenzymes the percentage ratio of H and M subunits was calculated (9) according to the formulas H% = LDH-5 + 0,75 LDH-4 + 0,5 LDH-3 + 0,25 LDH-2; M% = 100-H%.

Results

As it was already reported (7) LDH-activity in the leg lymph is remarkably high, exceeding 4 to 5 times its level in blood plasma. 30 minutes of muscular exercise produced already a significant increase in lymphatic LDH and the enzyme level was further raised during the next period of activity. This increase continued in the two observation periods following the end of the stimulation and finally a nearly 5 fold increase of LDH activity was registered in the leg lymph.

An increase in plasma LDH was first noted after 1 hour of muscular activity and 3 hour later in blood plasma the enzyme level was more than doubled. No significant difference could be detected between arterial and femoral venous blood LDH. In thoracic duct lymph initial LDH activity was a little below plasma activity and after the exercise a slow and gradual rise could be observed but the activity in lymph remained well below the activity in blood plasma.

Table 1 The effect of muscular activity on plasma and lymphatic LDH levels (mU/ml).

	1	2	3	4	5
Arterial plasma	26.6±3.5	26.7± 3.3	45.0± 7.9	61.0± 9.1	71.4± 8.7
Venous plasma	33.8±4.9	33.0± 4.9	53.8± 9.1	60.2± 5.9	70.4± 9.0
Thoracic duct lymph	21.1±4.3	22.2± 4.4	30.5± 5.0	31.4± 4.2	40.7± 4.6
Leg lymph	104.8±5.6	211.7±35.8	348.5±40.4	432.1±83.3	471.8±83.0

1 = Before muscle stimulation, 2 = after 30 minutes stimulation, 3 = after 60 minutes stimulation, 4 = one hour after the end of stimulation, 5 = three hours after the end of stimulation.

Between the LDH-isoenzyme patterns of serum and lymph a distinct difference was detected. The proportion of H subunits in serum was significantly higher than in thoracic duct or leg lymph ($p < 0.001$) (table 2). Muscle activity did however not lead to a

Table 2 Isoenzyme patterns in dog serum and lymph.

	LDH-1	LDH-2	LDH-3	LDH-4	LDH-5	H	M	H/M
A	45.6±3.5	20.7±1.4	19.7±1.9	7.0±1.0	7.9±2.0	72.1±2.7	27.9±2.7	2.82±0.33
V	45.6±3.5	19.5±2.3	17.1±2.2	5.8±1.5	12.1±3.1	69.7±3.8	30.3±3.8	2.89±0.51
L	17.3±2.0	21.8±3.3	37.9±2.9	14.1±2.9	8.9±2.5	57.0±2.9	43.0±2.9	1.44±0.15
D	22.0±2.2	22.3±2.4	36.3±2.5	14.0±2.1	4.8±1.0	60.7±1.6	39.3±1.6	1.62±0.11

A = arterial blood serum, V = venous blood serum, L = leg lymph, D = thoracic duct lymph.

Table 3 The effect of muscle activity on the proportional ratio of LDH-isoenzymes (per cent of H subunits).

	1	2	3	4	5
A	72.1 ± 2.7	70.0 ± 7.0	72.1 ± 3.7	67.2 ± 3.1	65.0 ± 3.8
V	69.7 ± 3.8	79.8 ± 2.4	75.2 ± 2.7	68.8 ± 3.1	67.6 ± 2.4
L	57.0 ± 2.9	59.7 ± 2.0	56.2 ± 3.0	57.2 ± 2.5	57.0 ± 2.6
D	60.7 ± 1.6	53.3 ± 3.1	56.9 ± 2.4	61.4 ± 3.7	63.5 ± 1.8

significant change in the isoenzyme patterns of serum and lymph. The calculated percentage ratios of H subunits in the serum and lymph samples collected before, during and after stimulation are presented in table 3.

Discussion

Exercise had increased LDH-activity not only in the lymph following from the active muscles but also in blood serum. Between the sera of arterial blood and of the venous blood of the stimulated extremity no significant difference could be detected. The increase of serum LDH was however rather small and the rise slow. Accordingly, a marked arterio-venous difference can hardly be expected.

In the present experiments the thoracic duct was cannulated i.e. the connection between the lymphatic and venous system was severed. The increase of serum activity is therefore an evidence for the direct access of the enzyme protein to the blood stream. Besides, the lymphatics of the stimulated extremity were cannulated or ligated and the LDH in the thoracic duct lymph remained consequently below the serum level.

Significant differences were detected between the isoenzyme patterns in serum and lymph. The higher ratio of M subunits in leg lymph could be explained by the tissular origin of the lymphatic LDH. In the muscle tissue of the dog, in accordance with similar observations made in other species, a high percentage ratio of M subunits was found (7). In the present experiments the H/M ratio was significantly lower than in blood serum also in thoracic duct lymph. This can be explained with the origin of thoracic duct lymph predominantly from tissues with a low H/M ratio (e.g. liver, intestines) but the possibility must also be taken in consideration that LDH with a high ratio of H subunits is released into the serum from the formed elements of the blood.

As it was already stated, no significant changes occured during or after muscle activity in the LDH-isoenzyme pattern of serum. The admixture of an equal amount of LDH with an isoenzyme pattern similar to that observed in leg lymph would however lead in serum to only about the same changes as were actually observed at the time when LDH activity in serum was approximately doubled. Compared to the control values these changes were not significant ($p > 0.10$).

Literatur

1 *Courtice, F. C.:* Lymph and plasma proteins: barriers to their movement throughout the extracellular fluid. Lymphology 4 (1971) 9-17

2 *Jepson, R. P., F. A. Simeone, B. M. Dobyns:* Removal from skin of plasma protein labeled with radioactive iodine. Amer. J. Physiol. 175 (1953) 443-448

3 *Loegering, D. J., J. B. Critz:* Effect of hypoxia and muscular activity on plasma encyme levels in dogs. Amer. J. Physiol. 220 (1971) 100-104

4 *Mayerson, H. S.:* The physiologic importance of lymph. In: Handbook of Physiology. Sect. 2. Circulation, Vol. II. (W. F. Hamilton Edit.) Amer. J. Physiol. 1963

5 *Schmidt, E., W. Schmidt, P. Otto:* Isoenzymes of malic dehydrogenase, glutamic oxaloacetic transaminase and lactic dehydrogenase in serum in diseases of the liver. Clin. chim. Acta 15 (1967) 283-289

6 *Selmeci, L., E. Posch, E. Balogh:* Effect of tourniquet on total lactate dehydrogenase (LDH) activity and isoenzyme pattern of rat serum. Acta physiol. Acad. Sci. hung. 38 (1970) 125-134

7 *Szabó, G., E. Anda, E. Vándor:* The lymphatic transport of lactate dehydrogenase. Effect of tissue anoxia and shock (in press)

8 *Szabó, G., Z. Magyar, G. Molnár:* Transport of macromolecules from the tissues. Proc. Int. Un. physiol. sci. 9 (1971) 549

9 *Thorling, E. B., K. Jensen:* The lactate dehydrogenase enzymes in various organs of the rabbit in anaemia, hypoxia and after cobalt administration. Acta path. microbiol. scand. 66 (1966) 426-436

10 *Van der Helm, H. J.:* Simple method of demonstrating lactic dehydrogenase isoenzymes. Lancet 1961/II, 108-109

11 *Vesell, E. S., A. G. Bearn:* Isoenzymes of lactic dehydrogenase in human tissues. J. clin. Invest. 40 (1961) 586-591

12 *Wieme, R. J.:* Application diagnostique de l'enzyme-électrophorèse des déshydrogenase de l'acide lactique. Clin. chim. Acta 4 (1959) 46-50

13 *Wieme, R. J., J. E. Herpol:* Origin of lactate dehydrogenase isoenzyme pattern found in the serum of patients having primary muscular dystrophy. Nature 194 (1962) 287-289

14 *Wright, E. J., L. P. Cawley, L. Eberhardt:* Clinical application and interpretation of the serum lactic dehydrogenase zymogram. Amer. J. clin. Path. 45 (1966) 737-745

15 *Wróblevsky, F., J. S. La Due:* Lactic dehydrogenase activity in blood. Proc. Soc. exp. Biol. Med. 90 (1955) 210-213

16 *Wróblevsky, F., C. Ross, K. Gregory:* Isoenzymes and myocardial infarction. New Eng. J. Med. 263 (1960) 531-535

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