Lymphology 7 (1974) 37-44

Lymphatic Transport of Enzymes After Experimental Myocardial Infarction

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Summary

The concentrations of LDH, MDH, GOT, creatine kinase, acid and alkaline phosphatase were examined in dogs in the cardiac lymph, arterial and coronary sinus blood serum prior to the occlusion of a descending branch of the left coronary artery and at 1, 2, 4, 6, and 24 hours thereafter. Lymphatic concentrations of LDH, MDH, GOT and CPK increased within the 1st to 2nd hour; serum levels rose later and remained below the lymphatic concentrations. 6 hours after the onset of the infarction there was a significant arterial-coronary sinus difference in the concentration of some enzymes. In serum a shift was observed in the LDH isoenzyme pattern in the direction of the preponderance of H subunits.

The changes in serum concentrations of the mitochondrial and lysosomal enzymes alkaline and acid phosphatase in the infarct animals did not differ from those observed in sham operated controls and their concentration in cardiac lymph remained below serum concentration.

The increase of the concentration of the cytoplasmic enzymes in cardiac lymph is the earliest sign of myocardial damage. The rise of serum levels of enzymes of cardiac origin in animals with cannulated cardiac lymph vessel and the presence of an arterio-venous concentration difference are positive evidences for direct venous transport of the enzyme proteins from the myocardium.

Ischaemic necrosis of the myocardium is not recognizable macroscopically before 12 to 16 hours after the occlusion of the coronary artery. No definite histologic signs can be observed before 6 hours, but there are biochemical evidences to an earlier onset of the tissue damage. The concentration of some intracellular enzymes rises in blood plasma at 6 hours, sometimes even as early as 2 hours after arterial occlusion (, 3, 8, 15, 16, 17). This biochemical alteration is not necessarily a sign of cellular breakdown or death, but may be the consequence of the increased permeability of the cell membranes (9, 11).

Macromolecular cell constituents, e.g. cytoplasmic enzymes, gaining access to the tissue fluid are transported by the lymphatic vessels. As tissue fluid from the myocardium cannot be collected, the first biochemical changes in a biological fluid accessible for study are to be expected in the cardiac lymph.

Material and Methods

General. The investigations were performed upon mongrel dogs anesthetized with pentobarbitone-sodium. The animals were divided into 4 groups.
Group I: After a right sided thoracostomy in the 4th intercostal space the cardiac lymph vessel (4), leaving the cardiac lymph node between the superior Vena cava and the right brachiocephalic artery was cannulated and lymph collected. Secondary lymphatic branches, present between the brachiocephalic and subclavian arteries were ligated. The thoracic cavity was then entered in the 4th left interspace, and the descending branch of the left coronary artery was ligated (10). At the operations, to avoid unnecessary tissue

damage, no ribs were resected and vessel ligations for haemostasis were reduced to the minimum.

After the coronary artery ligation the lymph collection was continued for another 6 hours. Arterial blood samples were taken before coronary ligation, and at 2, 4 and 6 hours afterwards. At the conclusion of the experiments the coronary sinus was cannulated through the right auricular appendage, and a venous blood sample was collected. To calculate coronary blood flow blood was allowed to flow freely for 1 minute from the cannulated sinus into a measuring cylinder.–

Group II: The coronary artery was ligated as in Group I, and there the thoracic cavity was closed. After 24 hours the thoracic cavity was re-entered on right side, cardiac lymph was collected, arterial and coronary sinus blood samples were taken, and the outflow from the coronary sinus was measured.

Group III (controls to group I): Bilateral thoracostomy was performed and arterial blood samples were taken as in group I, but the coronary artery was not ligated and the cardiac lymph vessel was not cannulated.

Group IV (Controls to group II): An arterial blood sample was withdrawn and a left side thoracostomy was performed as in group II. A second blood sample was obtained 24 hours after the operation.

Biochemical Methods

Malate dehydrogenase (MDH) was estimated in serum and lymph samples according to *Bergmeyer* (1). The results are expressed as mU/ml, corresponding in this as in all other enzymologic methods of our study to 1 nMol transformed substrate/ml/min at 25°C.
Creatine phosphokinase (CPK) activities were measured with the UV method of *Tanzer and Gilvang* (21). *Glutamic-oxalacetic transaminase* (GOT) was measured with the method of *Reitman and Frankel* (13).
Alcaline phosphatase activities (alc.P) of lymph and serum samples were measured according to the method of *Fischer and Siebert* (5).
Acid phosphatase was determined by the method of *Richterich* and al. (14).
Lactate dehydrogenase (LDH) was estimated according to *Wroblewsky and La Due* (24).

Lactate dehydrogenase isoenzymes were separated electrophoretically (23) in 1% agarose medium and sodium barbiturate-HCL buffer (pH 8,6; ionic strength 0,05) on microscopic slides at 7 V/cm. The slides were developed according to *Van der Helm* (22) and the percentage of isoenzyme fractions was determined with a Zeiss densitometer. From the distribution of isoenzymes the percentage ratio of H and M subunits was calculated.

Total protein concentration in serum and lymph was determined by the buiret method (7). Potassium was measured by flame photometry.

The values represented in the graphs and tables are averages with ± SEM. Statistical analyses of the differences were made with "Student's" t-test.

Results

In group I 18 dogs were operated upon. Five animals died within 6 hours after the coronary occlusion and were excluded from the study. One animal was not used because at autopsy no definite histologic signs of myocardial infarction could be detected. Five of the 14 animals of group II died before lymph collection could be performed.

Nine animals were studied. Control observations were made on 10 dogs in group III and 12 animals in group IV.

The average weight of the animals of both groups I and II was 14.7 ± 1.2 kg. Cardiac lymph flow in group I averaged 0.92 ± 0.11 ml/hour and coronary sinus outflow was 58 ± 12 ml/min. In the second group average lymph flow was 1.23 ± 0.11 ml/hr. The difference was not significant ($p > 0.05$).

The changes in enzyme concentrations in blood serum and lymph are shown in Fig. 1 to 6. As was reported previously (20) the LDH concentrations in cardiac lymph are significantly higher than in blood serum. The control values in group I were 36.5 ± 6.8 mU/ml on blood serum and 245 ± 34 mU/ml in the lymph. In the LDH isoenzyme pattern of cardiac lymph a preponderance of H subunits was detected (Fig. 7). H/M ratios were 1.60 ± 0.12 in normal serum and 2.67 ± 0.24 in lymph ($p < 0.05$).
After the occlusion of the coronary artery branch a small but steady rise in serum LDH concentration could be observed. The concentration change was statistically significant after 4 hours ($p < 0.01$). At 6 hours the LDH concentration in coronary sinus serum was significantly higher than the arterial concentration ($p < 0.05$).

In group II, 24 hours after coronary artery ligation, serum LDH activity was definitely higher than the control values (162 ± 26 mU/ml), but there was no significant arterio-venous difference. In the 24 hour controls (group IV) there was a small increase (to 54 mU/ml). The difference between the concentration changes in groups II and IV is significant ($p < 0.01$). In cardiac lymph the rise of LDH concentration was much greater than in serum, lymphatic concentration attained in the 4th to 6th hour 2360 ± 699 mU/ml. The increase of lymphatic concentration was significant already in the first hour ($p < 0.01$). 24 hours after coronary occlusion lymphatic LDH concentration was about the same as after 6 hours. In 6 hours there was a marked change in the isoenzymes in blood serum, with a significant increase in the H/M ratio. The isoenzyme pattern in coronary sinus serum was similar to that in arterial serum.

In the control experiments there was no significant change in serum LDH at 6 hours ($p > 0.05$).
MDH concentration changes in serum and lymph are parallel to those of LDH. In 6 hours MDH serum concentration rises in the infarct animals from 77 ± 12 to 108 ± 12 mU/ml. The increase is significant after 2 hours ($p < 0.05$). Twenty-four hours after coronary ligation there is about a fourfold increase in plasma MDH concentration. At 6 hours a positive arterio-venous difference was observed ($p \sim 0.05$). Normal MDH concentration is much higher in cardiac lymph than in serum (235 ± 19 mU/ml), and in 6 hours it rises to 3270 ± 1300 mU/ml. After 24 hours of coronary occlusion lymphatic MDH (3790 ± 535) is about the same as after 6 hours. In the controls (group III) there is a small, not significant rise in serum MDH ($p > 0.05$).
GOT. Serum concentration rises in six hours from 8.1 ± 1.9 to 13.2 ± 2 mU/ml ($p < 0.01$). The difference between the control and postinfarct values is significant ($p < 0.02$) after 4 hours. After 24 hours serum GOT reaches a value of 55 ± 2.9 mU/ml. At 6 hours there is a significant arterio-venous concentration difference ($p < 0.02$).

The concentration change in cardiac lymph was significant within the first hour and lymphatic GOT activity increased from 58 ± 24 mU/ml to 248 ± 63 mU/ml in 6 hours. In the animals with a 24 hour old infarct, lymphatic GOT was 289 ± 65 mU/ml. There was no significant change in serum concentration in the controls.

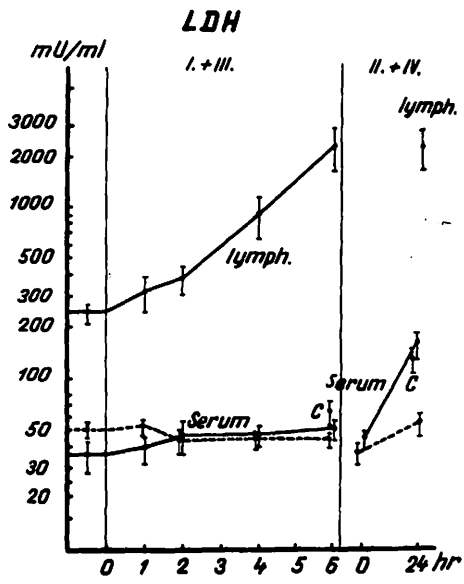

Fig. 1. Lactate dehydrogenase concentrations in cardiac lymph, arterial and coronary sinus blood serum before and after occlusion of the left coronary artery.

I. experiments with 6 hour occlusion of the coronary artery
II. experiments with 24 hour coronary occlusion
III. controls to group I
IV. controls to group II.

Solid curves: enzyme concentrations in arterial blood serum and cardiac lymph of the infarct animals.

Dashed curves: serum enzyme concentration in the control animals.

C: concentration in coronary sinus blood serum.

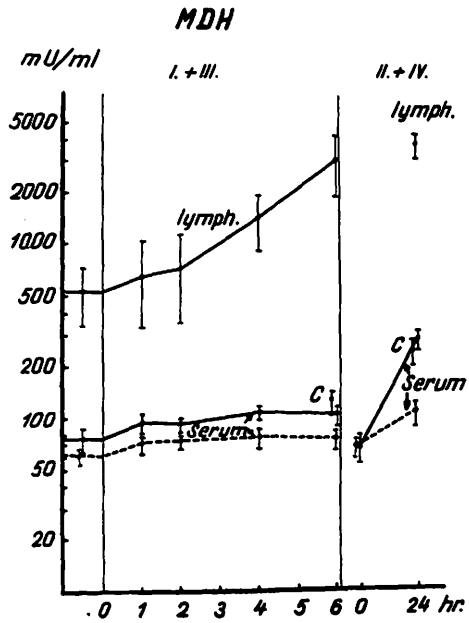

Fig. 2. Maleate dehydrogenase concentrations in serum and lymph before and after coronary occlusion.

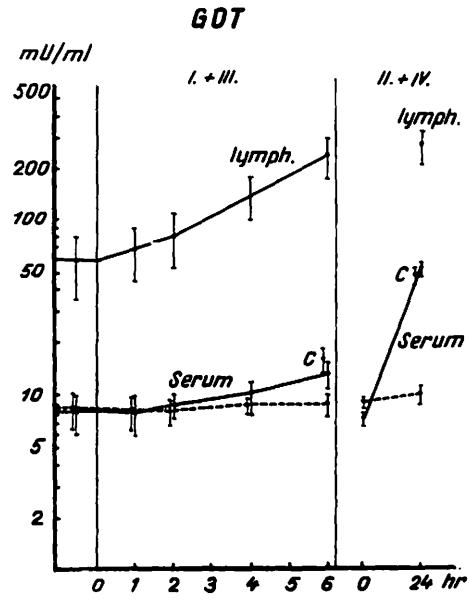

Fig. 3. Glutamic-oxalacetic transaminase concentrations in serum and lymph before and after experimental myocardial infarction.

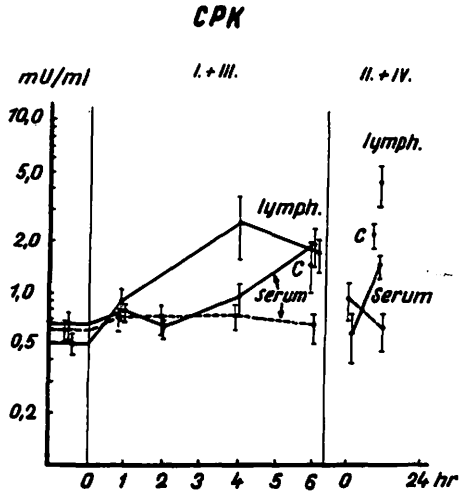

Fig. 4. Creatine phosphokinase concentrations in serum and lymph before and after occlusion of the coronary artery.

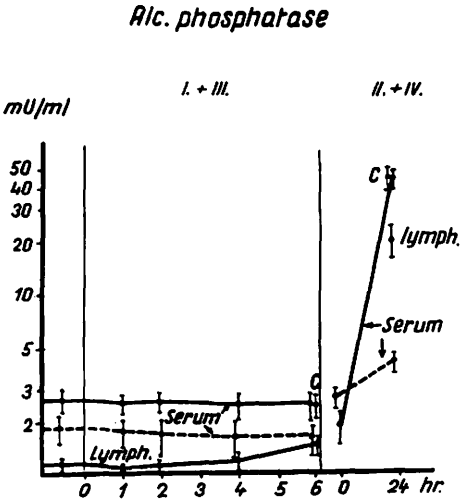

Fig. 5. Alkaline phosphatase concentration in serum and lymph in experimental myocardial infarction.

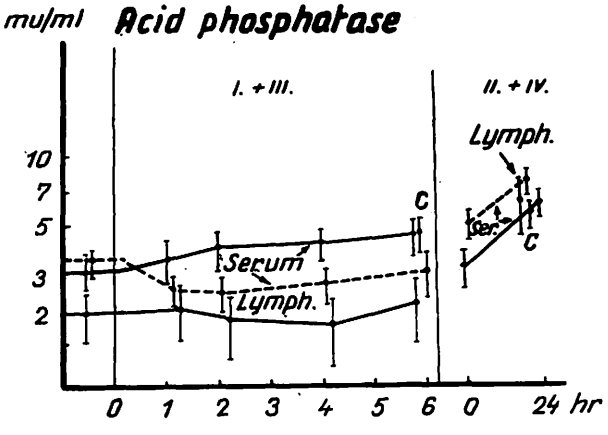

Fig. 6. Acid phosphatase concentrations in serum and lymph in experimental myocardial infarction.

CPK activity in serum rises in 6 hours from 0.66 ± 0.14 to 2.02 ± 0.54 mU/ml. The change is significant only in the 6th hour ($p < 0.05$). In group II (24 hr occlusion) arterial CPK was 1.6 ± 0.19 and the concentration in coronary sinus blood 2.4 ± 0.31 mU/ml ($p < 0.05$). The arterio-venous difference at 6 hours is not significant ($p > 0.50$). Lymphatic CPK concentration rises in 4 hours from 0.9 ± 0.2 to 2.7 ± 1.1 mU/ml ($p < 0.05$). In group II cardiac lymph enzyme concentration was 4.8 ± 1.3 mU/ml. In the conrols no change in serum CPK concentration could be observed.

Alcaline phosphatase concentration in serum did not change during the 6 hour observation period in the animals with coronary occlusion or in the controls. In the animals with a 24 hour old infarct serum phosphatase levels were higher than in both previous groups ($p < 0.01$). This enzyme is probably not of myocardial origin since there was no arterio-venous difference, the lymphatic enzyme levels remained below serum level and similar changes were observed in the comparable controls.

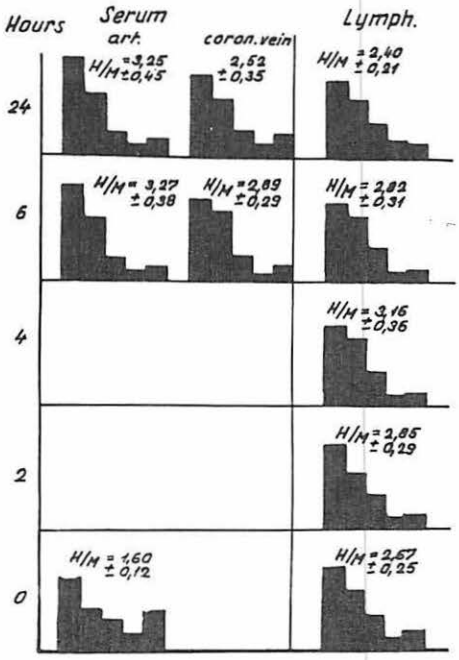

Fig. 7. Distribution of LDH-isoenzymes in arterial and coronary sinus blood serum and in cardiac lymph before coronary occlusion and 2, 4, 6 and 24 hours after the occlusion.

Acid phosphatase concentration rose in the serum during the 6 hour observation period (from 3.2 ± 0.55 to 4.6 ± 0.72 mU/ml, $p < 0.05$). The rise continued in the following hours and in the animals with a 24 hr old infarct, serum concentration attained 6.5 ± 0.88 mU/ml. Since there was no significant arterio-venous concentration difference, and since lymphatic concentration remained below serum concentration and since similar changes also were observed in the controls, the cardiac origin of this enzyme is dubious.

No connection was found between the changes in enzyme activities and plasma protein concentration. During the 6 hours of observation plasma protein concentration remained unchanged and lymphatic protein concentration rose only slightly.

Finally, potassium concentrations showed one interesting feature as evidence of cell breakdown : 24 hours after the infarct, potassium content of cardiac lymph was significantly higher than the concentration in blood serum (Table 1).

Table 1. Protein and potassium contents of arterial and coronary venous serum and of cardiac lymph before and after coronary artery occlusion

	protein g%			K^+ μEq/ml		
	arterial	venous	lymphatic	arterial	venous	lymphatic
0 hour (n = 11)	6.08 ±0.35		3.65 ±0.30	4.0 ±0.3		4.0 ±0.2
6 hours (n = 10)	5.85 ±0.29	5.55 ±0.25	4.00 ±0.29	3.6 ±0.3	3.5 ±0.3	3.8 ±0.2
24 hours (n = 9)	6.16 ±0.33	6.28 ±0.38	4.33 ±0.31	4.8 ±0.3	4.8 ±0.3	6.0 ±0.4

Discussion

After experimental myocardial infarction the concentration of the "cardiac" enzymes LDH, MDH, GOT and CPK rises in cardiac lymph before any change can be observed in blood plasma. Significant increases of lymphatic enzyme activity occurred as early as the first or second hour after ligation of a coronary artery branch. Corresponding rises of serum activity could be detected only after 2 to 6 hours. The normal, preinfarction enzyme conccentrations were (with the exception of CPK) significantly higher in cardiac lymph than in serum. This suggests that the enzymes are probably, in consequence of the cardiac activity, constantly released from the myocardium. This interpretation is sup-

ported by the fact that the only other tissue where lymphatic enzyme (LDH) concentration is higher than serum concentration is the skeletal muscle (20), and that the LDH content of the muscle lymph is further increased by muscle contractions (18).

The increases in enzyme concentrations noted after coronary occlusion were much higher in cardiac lymph than in serum.

The enzymes being discussed are "cytoplasmic", i.e. not associated predominantly with some cell component but present mainly in the supernatant fluid after cell fractionation. Accordingly, their presence in tissue fluid and lymph is not proof of cell breakdown, but may be due to the increased permeability of the cell membranes (9, 11). On the other hand, in our studies there has been no evidence of an escape of the mitochondrial or lysosomal enzymes from the damaged myocardium.

LDH, GOT, MDH, and CPK are not specific for myocardial tissue. High serum enzyme levels have been found after the operative procedures necessary for the production of experimental myocardial infarction (6, 12). This is obviously a consequence of the escape of the enzymes from the damaged (mainly muscle) tissue. In the present study, probably because the operations were less traumatic, serum enzyme levels in the control dogs did not rise or remained below the values observed in the infarct animals. The interpretation that after coronary ligation the increased serum levels are due mainly to enzyme release from the myocardium is supported by the changes in the LDH isoenzyme pattern. The proportion of H subunits in serum rose in 6 hours from 61 to 75% (H/M from 1.60 to 3.17). As a reference point it can be noted that in the dog the percentage of H subunits in heart muscle was found to be 75.7 (H/M 3.19) and in striated muscle H% = 46,3 (H/M 0.78)(20). The proportion of H subunits in arterial serum was even higher than in coronary sinus serum or cardiac lymph. This can be explained by the fact that the rate of disappearance from the circulation of the H-subunits is slower than that of the M-subunits (2).

One more important question merits consideration: the route by which the enzymes gain access to the circulation. It is generally believed that protein molecules are transported from the tissue fluid into the blood stream only by the lymphatic vessels. However, in the experiments where the connection between the cardiac lymph vessel and the venous system was interrupted, a significant increase of serum enzyme concentration developed nevertheless. The validity of this observations obviously depends on the evidence that the access of the cardiac lymph to the lymphatic system and hence to the venous system is indeed prevented. In some animals in these experiments, as well as in a previous study (19), Evans-blue dye or India ink was injected into the arterior wall of the left ventricle. It was established that no dye is transported by any other route except the cannulated cardiac lymph vessel. In addition to this indirect evidence, the presence of significant arterio-venous concentration differences is direct proof of the venous transport of the enzymes.

Calculations were made to define the ratio of venous versus lymphatic transport. In this study average cardiac lymph flow was 0.9 ml/hour, total coronary blood flow (from sinus outflow + correction for other venous channels) 65 ml/min. From this and the hematocrit average coronary plasma flow was calculated to be 2100 ml/hr. If these values are multiplied by lymphatic enzyme concentrations (in the samples collected between the 4th and 6th hr) and by the arterio-venous concentration differences, the venous/lymphatic transport ratios are 1.22 (LDH), 1.35 (MDH) and 2.5 (GOT). In a previous study (19),

with the injection of the labelled substances into the heart muscle, the venous/lymphatic ratio for ^{131}I-albumin was 3.9, for ^{125}I-PVP 3.8 and for ^{198}Au-colloid 0.77.

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