

Biochemical Changes in the Body Fluids after Ischaemic Tissue Injury*

G. Szabó and Zsuzsa Magyar

National Institute of Traumatology, Budapest, Hungary

Summary

Lymphatic and tissue fluid LDH activity increases significantly after 2 hr limb ischaemia but LDH content in tissue fluid is invariably higher than in lymph. The differences increase further after 4 hr ischaemia. The change of GOT concentration in the body fluids is a less sensitive indicator of tissue damage than the increase of LDH activity. A 2 or 4 hour ischaemia does not lead to rupture of lysosomes and to a marked activity increase of the lysosomal enzymes acid phosphatase and beta glucuronidase in tissue fluid and lymph. The earliest biochemical signs of cellular injury can be detected in the interstitial fluid of the damaged tissue.

During severe acute tissue injury there is an escape of intracellular electrolytes, metabolites and proteins from the damaged cells. Many or almost all of the escaped proteins are enzymes and can be detected by their specific activity in the extracellular fluids. The estimation of the activity of intracellular enzymes in circulating blood has been developed into a diagnostic method (5, 11, 12, 20). Actually cell constituents escape from the injured cells not into the circulating blood but into the interstitial fluid of the tissue. As the interstitial fluid itself is almost inaccessible the collection and examination of the regional lymph draining the interstitial space of the damaged tissue was proposed as a suitable approach (6, 7). It was actually shown, that in cats, dogs and rabbits the estimation of intracellular enzymes in the regional lymph after ischaemia, thermal or chemical injury of the limb, kidney or myocardium affords indeed a valuable method of assessing the extent of cellular injury (2, 3, 6, 7, 16, 18, 19).

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More recently a simple method has been proposed for the collection of small amount of tissue fluid (1) and it was shown, that both under normal conditions and after injury there are substantial differences in the concentration of various proteins in tissue fluid and lymph (15, 17).

Surgical interventions on the extremities, especially on the hand are often done under ischaemia, during a temporary interruption of the blood flow to the limb by means of tourniquet. The "ischaemia tolerance" of the tissues of the extremities is quite large. In rat-limb muscles irreversible changes appear only if total ischaemia lasts more than 4 hours (14). It would be of interest, nevertheless, to detect the earliest signs of ischaemic tissue damage and to follow its progress. In the present experiments intracellular enzyme activities were estimated in tissue fluid and regional lymph collected after complete limb ischaemia. As the intervention for tissue fluid collection provoked in muscle tissue almost invariably some haemorrhage and the collected fluid was contaminated with blood only observations made on subcutaneous tissue fluid and on lymph predominantly of cutaneous origin are reported.

Material and Methods

The experiments were done on rabbits of both sexes with body weights of 2.5 to 3.5 kg under pentobarbital anaesthesia (initial dose 30 mg/kg by intravenous injection). On both hindlimbs polythen cannulas were introduced in one of the medial afferent lymph vessels of the popliteal node. Lymph was collected by manually bending the leg in the knee

joint with a frequency of about 30 min. The afferent superficial lymph vessels of the popliteal node carry mainly cutaneous lymph (9). Cotton wicks, 4 to 6 cm long previously soaked in physiological saline were sewn into the subcutaneous tissue of the shanks. The wicks were pulled out after one hour and centrifuged under mineral oil. With this method about 0.02 to 0.05 ml of tissue fluid could be gained from each limb.

Leg ischaemia was produced by a broad elastic band fastened in the inguinal fold around the thigh. Only experiments were considered where the blood flow to the limb was totally stopped and during the 2 or 4 hour of ischaemia swelling of the limb or other signs of venous stasis were not observed. Lymph was collected in the 1st and 2nd and tissue fluid in the 2nd hour after the release of the tourniquet. Blood samples were withdrawn before limb ischaemia and 2 hours after its end. Control lymph and tissue fluid samples were obtained from the contralateral leg of the animal before tourniquet application.

In blood plasma, lymph and tissue fluid samples lactic acid dehydrogenase (LDH) activity was estimated according to *Wroblewsky* and *LaDue* (21), glutamic-oxalacetic acid transaminase (GOT) by the method of *Reitmand* and *Frankel* (2), acid phosphatase (AcP) according to *Richterich* et al. (11) and β -glucuronidase by the method of *Fishman* (4). The results presented in the text and the table are averages with \pm S.E.M. The statistical analysis is made with the paired *t* test.

Results

As it was shown previously (6, 7, 2, 13, 18) the increase of LDH activity in the biological fluids is the most sensitive indicator of cell injury. Already 2 hours after a 2 hr limb ischaemia there is a significant increase of LDH activity both in tissue fluid and lymph (s. Table). The change is greater in tissue fluid than in lymph and this difference is even more pronounced after a 4 hr ischaemia. At this time tissue fluid LDH activity exceeds about 10

times lymphatic activity. The other cytoplasmatic enzyme, GOT is a much less sensitive indicator of tissue damage. Its activity increased significantly only 2 hr after a 4 hr limb ischaemia, but the enzyme level was again sensibly higher in tissue fluid than in lymph. The lack of a marked rise in the lymphatic and tissue fluid levels of the two lysosomal enzymes AcP and β -glucuronidase shows that 2 or 4 hr ischaemic trauma is not so severe to provoke the rupture of an appreciable number of lysosomes in the affected tissues. A statistically significant minor rise of AcP activity was observed only in the tissue fluid after 4 hr ischaemia.

In blood plasma a significant rise of LDH activity was observed already after 2 hr. GOT activity in blood plasma rose only after a 4 hr ischaemia, but GOT plasma levels remained invariably below the enzyme levels observed in tissue fluid and lymph. There was no change in the concentration of the lysosomal enzymes in blood plasma.

Discussion

The degree of tissue injury depends obviously on the severity and duration of the trauma. The estimation of intracellular enzymes in the body fluids affords a method for the detection and for assessing the extent of cellular injury (2, 3, 6, 7, 16, 18, 19). One hour limb ischaemia seems to be only a mild trauma. In the cat, 1-2 hr after the release of the tourniquet only a moderate, non significant increase of LDH and GOT concentrations was observed in the regional lymph (6). Neither did the lymphatic concentration of lysosomal enzymes rise after a moderate thermal trauma (burning of the extremity at 50°, 60°, and 80°C) or in the rabbit at various intervals after a 4 hr limb ischaemia (3). Actually after the release of tourniquet, during the process of degeneration and regeneration lysosomal enzyme activities increased considerably in muscle tissue but their activity levels in the lymph from the paw were normal or below normal. A marked increase in the lymphatic activity of the lysosomal enzymes was observed only after the most severe injury, i.e. freezing the

Table The effect of limb ischaemia on the composition of tissue fluid and regional lymph

	N	Control limb		Ischaemic limb		Plasma	
		Lymph	Tissue fluid	Lymph	2 hr	Control	2 hr after tourniquet release
2 hr ischaemia							
LDH	9	179 ± 25	212 ± 31	215 ± 39	*343 ± 69	59 ± 11	96 ± 11
GOT	11	18 ± 2.5	14.3 ± 2.8	27.1 ± 3.7	*28.9 ± 3.9	15.5 ± 4.9	15.1 ± 3.5
β-glucur.	6	0.18 ± 0.04	0.36 ± 0.15	0.22 ± 0.03	0.32 ± 0.05	0.55 ± 0.08	0.67 ± 0.04
AcP	5	25 ± 5	25 ± 2	26 ± 5	26 ± 6	42 ± 2	40 ± 1
4 hr ischaemia							
LDH	9	200 ± 48	401 ± 41	*769 ± 251	3904 ± 1870	52 ± 5	**1136 ± 188
GOT	5	25.5 ± 3.3	21.0 ± 4.4	24.2 ± 2.3	*59.5 ± 3.9	12.5 ± 1.6	46.7 ± 7.1
β-glucur.	14	0.15 ± 0.07	0.44 ± 0.04	1.14 ± 0.02	0.19 ± 0.05	0.58 ± 0.05	0.64 ± 0.07
AcP	13	24 ± 5	23 ± 3	23 ± 4	26 ± 5	45 ± 4	41 ± 5

*Significant change $p < 0.05$;** $p < 0.01 - 0.001$

limb solid (6). In the present study after 2 and 4 hr ischaemia there was in tissue fluid and lymph an activity increase of the cytoplasmic enzymes, especially of that of LDH. The concentration of the lysosomal enzymes did change but little and the increase was significant only in the case of AcP in the tissue fluid after 4 hr ischaemia. The tissue fluid and lymph studied was of cutaneous origin and this tissue is relatively rich in lysosomes. The finding that lysosomal enzymes did not appear in the regional lymph and tissue fluid in any appreciable amount shows that at 2 or 4 hours arrest of blood flow to the limb is not a massive injury provoking a rupture of the lysosomes and release of their enzymes into the extracellular fluid. This observation might be explained by the assumption that during the early period there is no lysosomal rupture and the lysosomes are involved only in the postnecrotic phase, i.e. in the later scavenging processes (13). On the other hand, it was observed that in the period of cell degeneration, when its level of activity was highest in the injured tissue the β-glucuronidase activity was significantly decreased in the regional lymph. It has been suggested, therefore, that the enzyme in tissue fluid which originates from the blood plasma is involved in the lytic process of the damaged cells (3). The fact that a massive injury, e.g. freezing the tissues of an extremity solid (6) or a 2 hr renal ischaemia (16) leads to an early release of lysosomal enzymes speaks in favor of the assumption, that the rupture of the lysosomes depends on the severity of the trauma.

The question should be raised, consequently, whether the escape of cell proteins into the tissue fluid is a sign of cellular breakdown or it may occur already after a mild injury from cells remaining functional but undergoing transient changes in permeability or metabolism (8, 20).

The latter assumption is supported by the finding that the biochemical changes in the body fluids differ according to the severity of injury not only quantitatively but also qualitatively. It was shown, that the amount of cytoplasmic enzymes in lymph and tissues fluid increases progressively with the

severity of injury and on the other hand, lysosomal enzymes accumulate in the extracellular fluids only after massive tissue damage (6, 16 and the present study).

The recent observations corroborate the earlier findings (17) that there are marked differences in the biochemical composition of tissue fluid and of lymph proving from the same region. On the other hand, the estimation of cellular enzymes in circulating blood is a well established method for the diagnosis of acute tissue injury (12, 15, 20). The present and earlier findings have shown that in respect of the time of appearance of the biochemical changes in the body fluids there is a definite sequence. The cellular enzymes accumulate first in tissue fluid, than appear in regional lymph and last comes the rise of their concentration in circulating blood. In the severity of the changes the same order of succession can be observed. Accordingly, the signs of cell damage should be, whenever possible, sought for first in the tissue fluid of the injured organ.

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Dr. G. Szabó, Traumatology Research Group, P.O.B. 370, Budapest 1445, Hungary