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The Relationship between Tissue Fluid and Lymph II. Enzymes in Tissue Fluid and Lymph

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Summary

In rabbits both under normal conditions and after burning the activity of intracellular enzymes (LDH, GOT) is higher in tissue fluid than in the peripheral (leg) lymph. The relationship between tissue fluid and lymphatic enzyme concentrations is non-linear. The observations suggest the presence of at least two distinct compartments in tissue fluid. The first compartment forms the pathway taken by the fluid and protein leaving the blood capillaries and is directly drained by the lymphatics. The second compartment is the 'true' tissue fluid, contains the extravascular protein pool and it is in dynamic equilibrium with the first compartment.

It is generally accepted, that lymph and tissue fluid are identical, the lymph actually represents a cross section of tissue fluid in the area concerned (4). Furthermore, it is assumed that colloid molecules present in tissue fluid, e. g. plasma proteins escaping from the capillaries, the products of cell secretion or cleavage etc. are transported from the intestinal space only by the lymphatic vessels (3, 8). Recently evidence has been presented, that neither of the above views is exactly true, i. e. qualitative and quantitative differences have been detected in the composition of tissue fluid and lymph (13, 15) and it was shown, that colloid molecules may be absorbed from the tissues both by lymphatics and blood capillaries (10, 11, 12, 14). The above observations make a revision of the current views on the relationship between tissue fluid and lymph necessary.

The concentration of some intracellular enzymes in peripheral lymph is relatively high, usually higher than in blood plasma. Excessively high lymphatic enzyme levels are observed after injuries involving cell damage (2, 5, 6, 7, 10, 11, 14, 16). It was concluded therefore that the estimation of intracellular enzymes in the lymph draining an injured tissue affords a method of assessing the extent of cellular injury (5, 6). The enzymes are, however, released from the damaged or undamaged cells into the tissue fluid, not into the lymph. It is of highest importance to gain information on the enzymes in tissue fluid and to compare lymphatic and tissue fluid enzyme activities.

Material and Methods

Experiments were performed on rabbits anaesthetized with pentabarbitone (30 mg /kg) given intravenously. A lymph vessel was dissected along the line of the saphenous vein and cannulated immediately below the popliteal node with a polyethylen cannula. The lower abdomen was opened by a midline incision, the posterior peritoneum was incised along the lower borders of the m. psoas minor and the lumbar trunks were dissected out and cannulated. Lymph was collected, usually in 15 min periods, by manually bending the leg in the knee joint with a frequency of about 30/min. Cotton wicks previously soaked in physiological saline solution, 4 to 5 in number and each 4 to 6 cm long were sewn into the subcutaneus tissue of the shank and were allowed to equilibrate with tissue fluid (1). After 1 hour the wicks were pulled out and centrifuged under mineral oil for 30 min at 8000 rev/min. With this method 0.02 to 0.05 ml tissue fluid could be gained from each limb.

Tissue injury was produced by immersing the hind limb, usually the right of the animal for 15 to 20 sec up to the groin in water with a temperature of 80°C. In the these experiments the cotton wicks were introduced 30 minutes after burning and pulled out at 90 min. Leg lymph collection in the injured extremity was started 1 hour after burning

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and lasted about 15 min. Lumbar trunk lymph was collected after the conclusion of the leg lymph collection. Lymph and tissue fluid from the uninjured extremity was collected in the first hour after burning. Arterial blood plasma was obtained 1 hour after the scalding of the extremity.

Lactic acid dehydrogenase (LDH) in tissue fluid, lymph and plasma samples was estimated by the U. V. method of *Wroblevsky* and *La Due* (17), glutamic-oxaloacetic acid transaminase (GOT) by the method of *Reitman* and *Frankel* (8), total protein by the biuret method or by micro-*Kjeldahl* destillation.

The results in the text and tables are averages ± s. e. m. The statistical analysis is made with the paired t test, or the t test for the comparison of two means.

Results

In 7 normal rabbits, as seen in previous studies (10, 16), LDH activity in leg lymph was significantly ($p < 0.05$) higher than in blood plasma. Lumbar trunk lymph contained, however, significantly less LDH than the leg lymph. On the other hand, tissue fluid LDH was nearly 10 times higher than the enzyme activity in blood plasma, and about twice as high as the activity in the leg lymph (Table 1). After burning (9 animals) tissue fluid and lymphatic enzyme activities attained very high levels, but tissue fluid contained about 8 times more LDH than leg lymph. The LDH activity in the lumbar trunk lymph was on the other hand much higher than in leg lymph (Table 2). The high LDH in the postnodal, lumbar trunk lymph derives obvicusly from the damaged lymphoid cells. In the uninjured limb of the same animals tissue fluid and leg lymph LDH activities were about the same as the enzyme activity in blood plasma. Lumbar trunk lymph collected from the uninjured side contained very much LDH and it can be assumed that it was probably contaminated through cross anastomoses with lymph from the other side (Table 2).

Table 1 Tissue fluid and lymph LDH and total protein in 7 normal rabbits.

	LDH U/ml	Total protein g/dl
Plasma	29 ± 3	6.09 ± 0.34
Leg lymph	151 ± 28	2.41 ± 0.12
Lumbal tr.lymph	88 ± 14	2.56 ± 0.21
Tissue fluid	274 ± 28**	3.56 ± 0.15*

*; **: Significant difference between tissue fluid and lymphatic concentrations (*: $p < 0.05$; **: $p < 0.01$)

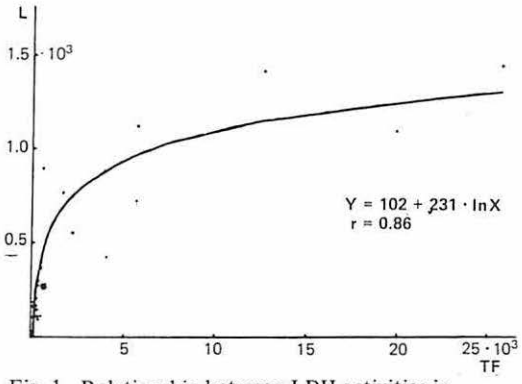

Fig. 1 Relationship between LDH activities in tissue fluid and prenodal leg lymph in normal and burned rabbits.

The tissue fluid and lymphatic GOT activities in the burned and control legs showed a pattern similar to that of LDH, only GOT activities did rise much less spectacularly.

When the enzyme activities in the tissue fluid and in the prenodal (leg) lymph originating from the same region were compared, a non linear relationship was observed. The best fit with a high coefficient of correlation ($r = 0.86$) was obtained with a logarithmic regression line (Fig. 1).

In accordance with previous observations (10, 16) in normal animals total protein concentration was significantly higher ($p < 0.01$) in tissue fluid than in leg lymph. Lumbal trunk lymph contained about as much protein as leg lymph. In the burned animals both tisssue fluid and lymphatic

Table 2 Protein and enzyme concentration in tissue fluid and lymph in animals subjected to scalding with hot water (15–20 sec on 80 °C).

	LDH U/ml		GOT U/ml		Total protein g/dl	
	C	I	C	I	C	I
Plasma	282 + 30		43 + 7		5.39 + 0.22	
Leg lymph	322± 30	928 ± 120**	54±14	127 ±12**	2.62±0.28	3.77**±0.59
Lumbal tr.lymph	660±250	2628 ± 920**	105±19	170 ±36*	2.90±0.42	3.81**±0.30
Tissue fluid	281± 45	7435**±2967*	60± 5	280*±45**	2.21±0.20	4.19**±0.42

C: control, uninjured eytremity; I: burned extremity
*; **: significant difference compared to the control value (*: $p < 0.05$; **: $p < 0.01–0.001$)
x: significant difference between tissue fluid and lymphatic (leg and lumbar trunk) concentrations (p 0.01–0.001).

protein concentrations were significantly higher ($p < 0.05$) than in the normals. In these animals no significant differences were seen between the protein content of the tissue fluid, leg lymph and lumbar trunk lymph, respectively. In the uninjured hind limbs of the same animals tissue fluid total protein concentration was significantly ($p < 0.05$) lower than in normal animals. Lymphatic (leg and lumbar trunk lymph) protein concentrations were about the same as in the normal animals. Actually in the intact extremity of the burned animals no significant difference was observed between tissue fluid and lymph protein concentration. Between the pooled total protein concentrations of tissue fluid and leg lymph the relationship was linear, but the coefficient of correlation (0. 46) was only moderate. All but one data from the intact extremity of burned animals fell below the regression line (Fig. 2). However, in a similar study made in over 30 normal rabbits the correlation between tissue fluid and lymph protein concentration was not much higher ($r = 0.52$). (15)

Discussion

The present study confirms the observation (10, 14, 16) that in some tissues intracellular enzymes are continuously released into the lymph. It was also shown, that the enzyme activities are markedly higher both in normal and pathological conditions in the subcutaneous tissue fluid than in the lymph originating from the same region. After burning, when the enzyme release from the damaged cells is high, the relationship between tissue fluid and lymph LDH is non-linear. From the regression equation it can be roughly calculated that when tissue fluid LDH is, doubled, (e. g. from 5000 to 10000 units) lymphatic LDH increases only by 160 units. Studies on the protein content of tissue fluid and of lymph (13, 15) suggested a sieving action of the wall of the initial lymphatics or a restricted passage of the colloid molecules accross the connective tissue matrix. On the other hand, when the equilibration of intravenously injected radioalbumin was studied (15) no significant difference was observed between tissue fluid and lymph, i. e. the equilibration of the labelled protein in the lymph was not considerably delayed. These observation suggest a complex structure of the tissue fluid, the presence of at least two distinct compartments.

The first compartment is connects the blood capillaries and the lymphatics and forms the pathway taken by the fluid and protein leaving the blood capillaries. The second is a pool not directly drained by the lymphatics. The first compartment is the fluid on its way from the capillaries to the initial lymphatics and in the isovolumetric state of the extremity its volume and composition is roughly equal to the volume and composition of the fluid leaving the capillaries. The second compartment is the true tissue fluid. It contains the extravascular plasma protein pool and the protein molecules

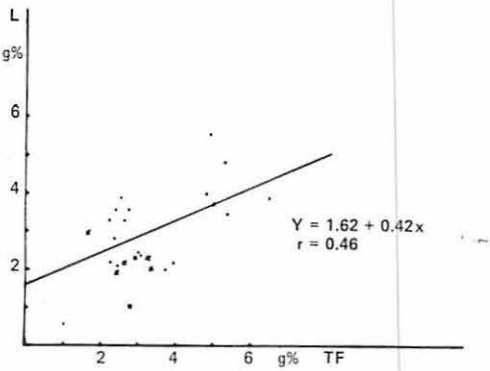

Fig. 2 Relationship between total protein concentration in tissue fluid and leg lymph in normal and burned animals (x: control legs of the burned animals).

released from the cells and is in a dynamic equilibrium with the first compartment.

The above hypothesis is supported by some other observations made in the present investigations. In the burned extremity, where the permeability of the blood vessels to protein is markedly increased and fluid leakage from the damaged capillaries and consequently lymph flow is high, the interstitial space is flooded by a fluid with a very high protein content. The mean protein concentration of the leg lymph and subcutaneous tissue fluid approaches 75 and 80 per cent respectively of the plasma protein concentration and there is no significant difference between lymph and tissue fluid. The escape of large amounts of plasma protein into the damaged tissue decreases protein concentration in the blood plasma and consequently mobilizes protein from the extravascular pool of the undamaged tissues. This is reflected by the low protein content of tissue fluid in the control, unburned leg of the animals. In the burned animals the protein concentration in the fluid obtained from the undamaged subcutaneous tissue was the same as in the skin lymph collected from the regional lymphatic.

It can be concluded, that intracellular enzymes, or generally proteins of cellular origin are released into a relatively stationary compartment of the tissue fluid, the "true tissue fluid". Plasma proteins escape from the blood capillaries to a mobile part of the extravascular fluid, the "capillary filtrate"; which is drained by the lymphatics. The two compartments are in dynamic equilibrium and changes in their composition are reflicted in the lymph.

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