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Enzymes in Tissue Fluid and Peripheral Lymph

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Summary

Enzymes escaping from the tissue cells (e.g. LDH, GOT, GPT) are present in the regional lymph often in higher concentrations than in blood plasma. This proves only the lymphatic transport of the enzyme proteins but does not exclude the possibility of their direct entry into the blood capillaries. In pathological conditions e.g. after burning or tissue ischaemia when the release of cellular enzymes is increased the enzyme activities increase markedly in the regional lymph but in many organs the direct entry of the enzyme molecules into the blood stream is also evidenced by a significant arterio-venous concentration gradient. In some cases the venous transport may be even much more important than the lymphatic one.

The enzymes are released from the cells not into the lymph but into the tissue fluid. It was shown that in the subcutaneous tissue fluid enzyme concentrations are normally higher than in the regional lymph. This difference increases markedly after tissue injury. Tissue fluid also contains more plasma protein than lymph. Based on the above observations a two compartment system of the tissue fluid has been proposed. The first compartment is the pathway actually taken by the fluid and protein leaving the blood capillaries and entering into the lymphatics. The second compartment is a pool not directly drained by the lymphatics and it represents the true tissue fluid.

Enzymes formed in the exocrinic glands are normally directly released into the secretion of the organ. E.g. proteases, lipases, carbohydrases of the gastrointestinal tract are secreted into the digestives juices, phosphatase and hyaluronidase formed in the male reproductive organs are released into the seminal fluid. Some enzymes, e.g. plasma lipoprotein lipase and other esterases, coeruloplasmin etc. seem to be released directly into blood plasma. Under pathological conditions, e.g. after the occlusion of the pancreatic duct, there may be a high enzyme concentration in the regional lymph but this is a secondary consequence of the enzyme leakage from the intraorganic ducts.

On the other hand, intracellular enzymes are in consequence of cell cleavage, damage or destruction released into the extracellular fluid. A proof for the continuous enzyme release from the cells is their presence in blood plasma. The enzymes are proteins. It is generally assumed that the proteins of the tissue fluid, irrespective of their origin, i.e. plasma proteins excaped from the blood capillaries or enzyme proteins released by the tissue cells, are carried away by the lymph vessels. On the other hand, according to the time honored concept the composition of the lymph and of the extracellular fluid is identical. The lymph represents a cross section of the tissue fluid in the area concerned. Actually neither of these two propositions is exactly true.

Let us see first the signs for the release and lymphatic transport of intracellular enzymes. In a tissue the most reliable sign for the formation or release of a specific protein is its higher concentration in regional lymph than in arterial blood plasma. In most cases a significantly higher L/P concentration ratio for a specific protein than for plasma albumin or total protein might be accepted as a proof for its release and lymphatic transport.

This problem was approached by the study of the lymphatic transport of LDH in several organs (1). It was observed that cardiac lymph and the lymph collected from the femoral lymph vessels have significantly higher LDH activities than blood plasma (Fig. 1). In the case of LDH there is a further possible evidence for the origin of the enzyme from a specific tissue. As it is well established LDH has 5 isoenzymes which are formed by the combination of 2 genetically determined subunits designed as H and M. The isoenzyme pattern is organ specific. Comparing the distribution of isoenzyme fractions in an organ (i.e. tissue homogenate) and their pattern in the regional lymph and blood plasma, respectively it was established, that

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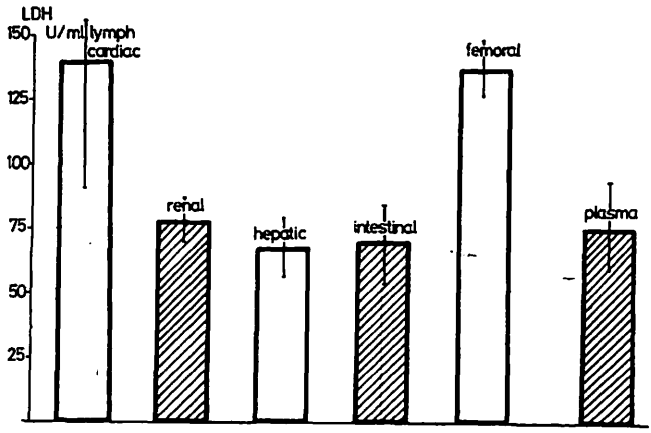

Fig. 1 LDH activities in regional lymph and blood plasma in normal dogs

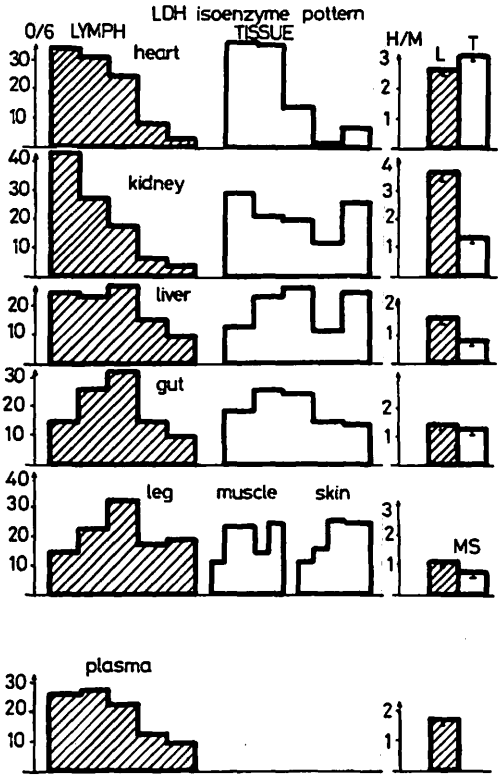

Fig. 2 LDH isoenzyme patterns and H/M ratios in regional lymph, tissue homogenisate and blood plasma in the dog.

lymphatic and plasma isoenzyme patterns or H/M ratios were identical only in the liver (Fig. 2). In the heart and the gut the lymphatic LDH isoenzyme distribution was similar to the pattern observed in the tissue homogenate. The isoenzyme distribution in femoral lymph was similar to the pattern in skin and muscle tissue of the leg (Fig. 2). These observations may be considered as sign for the lymphatic transport of LDH of tissular origin. In a few selected tissues the lymphatic concentrations of some other enzymes were investigated as well (2, 3). In cardiac lymph not only LDH L/P concentration ratio was significantly above unity, but also that for MDH and GOT (Fig. 3). On the other hand the acid and alcaline phosphatase activities were in cardiac lymph lower than in blood plasma.

In renal lymph only MDH concentration was significantly higher than plasma concentration, for LDH and GPT only the L/P ratios were significantly higher than the ratio for total protein (Fig. 4). Finally in liver lymph GOT activity was significantly higher than in blood plasma (4). Alcaline phosphatase concentration in hepatic lymph was significantly lower than in blood plasma, and remained well below it at the very high plasma phosphatase activities observed after common bile duct obstruction. This indicates that the enzyme which is of hepatic origin is directly released into sinusoidal blood and not into tissue fluid and lymph.

Lymphatic concentration higher than plasma concentration does only prove that the enzyme is transported by the lymphatics but does not exclude the possibility of its entry into the blood capillaries and transport by the veins. A positive proof for the venous transport of a

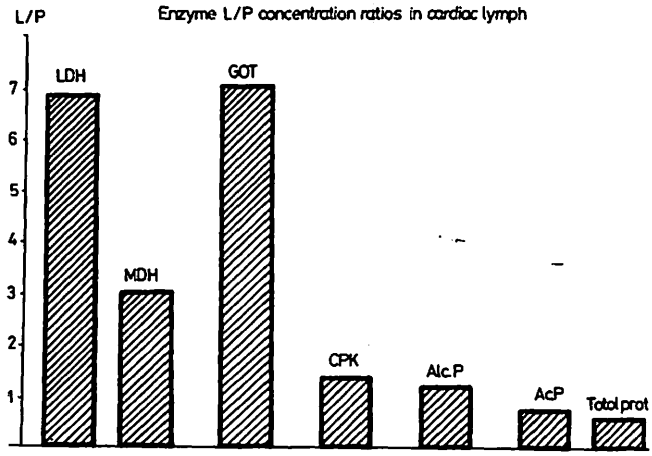

Fig. 3 Enzyme lymphatic/plasma activity ratios in cardiac lymph (normal dog).

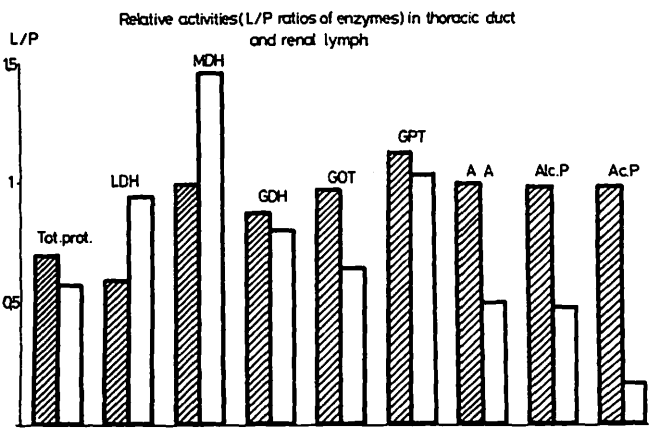

Fig. 4 Relative activities (L/P ratios) of various enzymes in thoracic duct (shaded columns) and renal lymph (white columns).

protein can be obtained by showing, that in the venous efluent of the organ its concentration is higher than in arterial blood, or by demonstrating that its concentration in blood plasma rises after the ligation or cannulation of all lymphatics of the organ and/or of the thoracic duct and right lymph trunk. By the latter method the entry into the blood capillaries of intratissulary injected labelled protein could be demonstrated in several tissues (5).

In dogs after a transitory renal ischaemia produced by clamping of the renal arteries the plasmatic concentration of several enzymes rises even if the lymph is diverted from the blood stream by the cannulation of the thoracic duct. More important still, after a 2 hour renal ischaemia a highly significant renal arterio-venous LDH concentration difference was observed (4).

On the other hand 24 hours after the ligation of a coronary artery branch a significantly higher LDH, MDH, GOT and CPK concentration was observed in coronary venous than in arterial blood plasma. The activity of the same enzymes in cardiac lymph was markedly increased. There was no change in lymphatic acid and alcaline phosphatase concentration, and there was no arterio-venous difference in the concentration of these two enzymes.

The lymphatic and venous transport of LDH was in dogs studied also after regional muscle ischaemia (6). The blood flow to the hind limbs was occluded for 3 1/2 hours. After tourniquet release LDH activity in leg lymph in-

creased markedly. At the same time a significant difference was detected between the LDH activity in v. femoralis and arterial blood plasma. The thoracic duct was cannulated in these animals and yet arterial blood LDH activity increased. This observation as well the significant arterio-venous concentration difference can be interpreted as a sign of direct entry of the enzyme into blood circulation.

The venous transport of the enzymes from the tissues as compared to the lymphatic transport is by no means negligible. From the experimental data we have calculated that in the skeletal muscle more LDH is transported by the venous than by the lymphatic route.

The venous/lymphatic transport ratio is in the kidney for LDH 60, for GOT 94. In the myocardium it varies between 1.22 for LDH and 2.5 for GOT (7). In other words, in the kidney nearly 100 times more GOT enters directly into the blood capillaries than into the lymph vessels. This values are in very good agreement with observations made after intratissular injections of labelled albumin.

The entry of the enzyme molecules into the blood capillaries is a diffusion process. Accordingly the ratio of venous versus lymphatic transport depends from the structure of the capillary wall, the presence of open junctions, fenestrae or interendothelial gaps. There are therefore great differences in the ratios observed in the individual tissues. Furthermore the ratio depends from the molecular size of the individual enzymes and from the tissue fluid – plasma concentration gradient. The calculated values are valid only in presence of a high concentration gradient, for the case when plasma concentration compared to tissue fluid enzyme concentration is practically negligible. It should be remembered that in this situation, i.e. when there is a massive release of intracellular enzymes in consequence of cell damage or destruction, e.g. after ischaemia (6) or burning of the extremity (8, 9, 10),after partial myocardial ischaemia, or temporary renal ischaemia, the lymphatic enzyme concentration is very high, it actually exceeds several times the concentration in blood plasma.

All informations in so far related are about the lymphatic concentration of the enzymes. But if the concept that lymph and tissue fluid are identical is accepted the data about the lymphatic concentration give also information about the enzyme release into tissue fluid. Recently, however, evidence has been presented, that there are qualitative and quantitative differences in the protein composition of lymph and tissue fluid (11). The enzymes are on the other hand released from the damaged or undamaged cells into the tissue fluid and not into the lymph. It was of highest importance to gain information on the intracellular enzymes

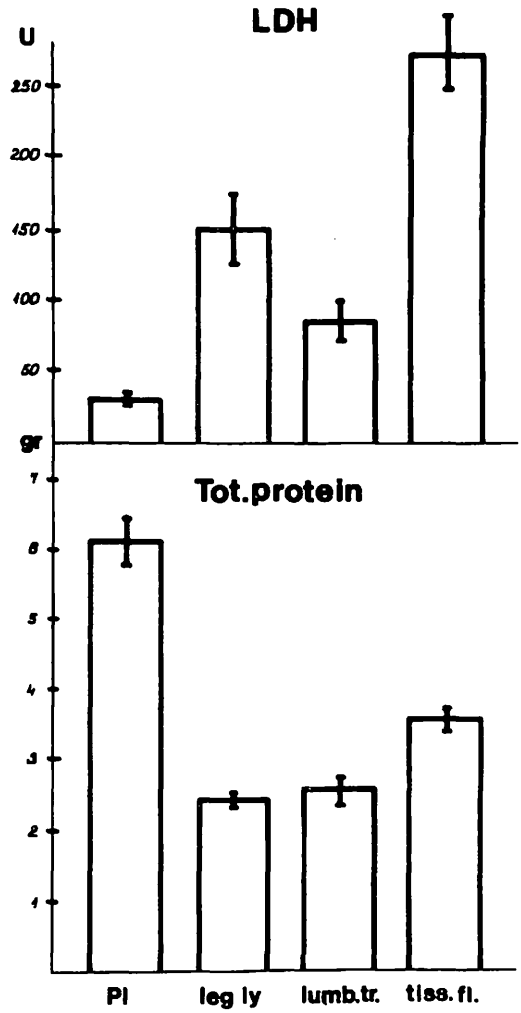

Fig. 5 LDH activities and total protein concentrations in the crural and lumbar trunk lymph, tissue fluid and blood plasma of normal rabbits.

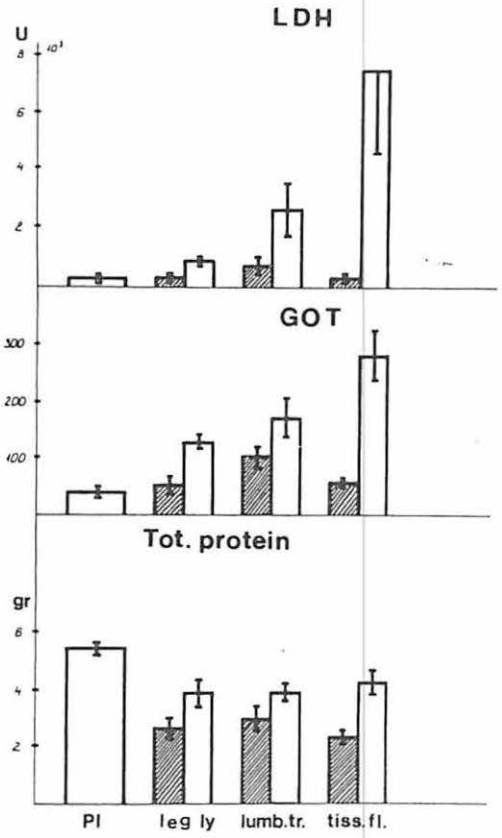

Fig. 6 LDH and GOT activities and total protein concentration in blood plasma, lymph and tissue fluid of rabbits after burning. (White columns: lymph and tissue fluid samples from the burned leg, shadded columns: unburnt extremity of the same animals.)

in tissue fluid itself and to compare lymphatic and tissue fluid enzyme activities.

The studies were made in rabbits. Tissue fluid was collected from the leg of the animals with cotton wicks sewn into the subcutaneous tissue of the shank and removed after 1 hour. With this method 20 to 50 μl tissue fluid could be obtained from each limb. Prenodal peripheral lymph was collected by cannulating below the popliteal node a lymph vessel running along the saphenous vein. Postnodal lymph was collected from the lumbar trunk. Lymph was collected in 15 min periods by manually bending the extremity in the knee joint with a frequency of about 30/min. Tissue injury was produced by immersing one hind limb of the animal for 15 sec into hot water with a temperature of 80 °C. In these experiments the cotton wicks were introduced 30 min after burning and pulled out after 90 min. Leg lymph collection started 1 hr after burning. Lumbar trunk lymph was collected after the conclusion of leg lymph collection. Lymph and tissue fluid from the uninjured extremity was obtained in the first hour after burning.

In the prenodal leg lymph of normal animals LDH activity was again significantly higher than in blood plasma (Fig. 5). Lumbar trunk lymph contained, however, significantly less LDH than the crural lymph. In the rabbit the lymphatics running along the saphenous vein carry almost exclusively cutaneous lymph and the lumbar trunk collects the lymph from both the superficial and deep lymphatic vessels

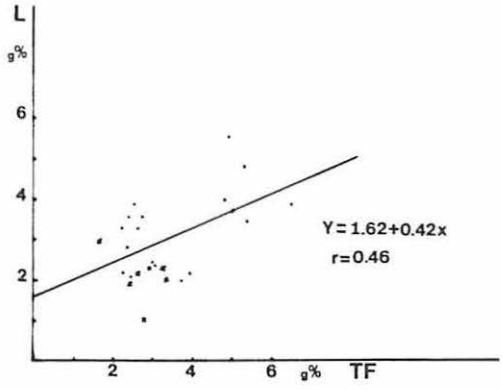

Fig. 7 Relationship between lymphatic and tissue fluid protein concentrations in burned rabbits.

L: lymph collected from a prenodal crural lymphatic.
TF: tissue fluid collected from the shank of the same animals
x: data from the intact extremity.

of the extremity. Tissue fluid LDH was nearly 10 times higher than the enzyme activity in blood plasma and about twice as high as in leg lymph (Fig. 5). In accordance with previous observations total protein concentration was significantly higher in tissue fluid than in leg lymph. Lumbar trunk lymph contained about the same ammount of protein as leg lymph.

After burning tissue fluid and lymph enzyme activities attained very high levels, but in the

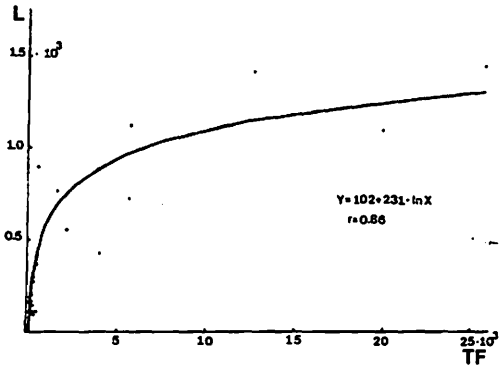

Fig. 8 Relationship between lymphatic and tissue fluid LDH activities in the leg of the rabbit.

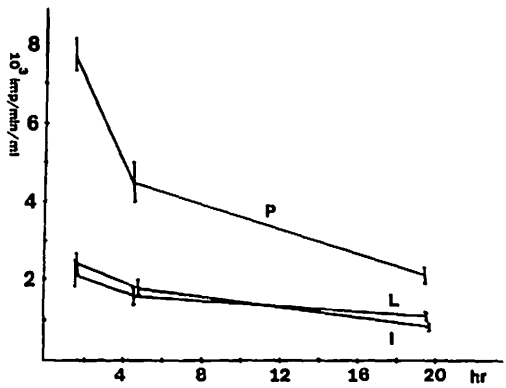

Fig. 9 Equilibration between blood plasma (P) and lymph (L) or tissue fluid (I) of intravenously injected radioactive albumin in the rabbit.
a) counts per unit volume of fluid

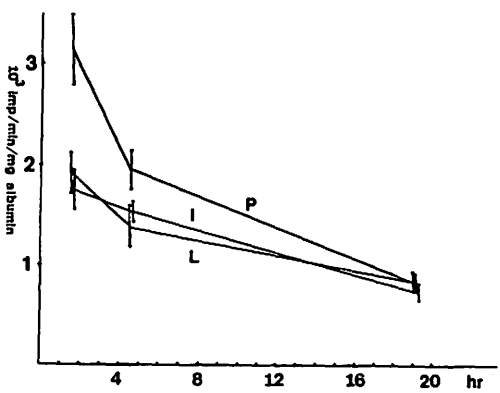

Fig. 9b counts per unit mass of albumin.

burned extremity tissue fluid contained about 8 times more LDH than leg lymph. The LDH activity in lumbar trunk lymph was much higher than in the crural lymph. The excess LDH in the postnodal lumbar trunk lymph derives probably from the damaged cells of the lymph nodes. In the uninjured limb of the same animals tissue fluid and leg lymph LDH activities were about the same as the activity in blood plasma (Fig. 6). It must be mentioned, that in these animals plasma activities were much elevated, to about 10 times the normal control value. Lumbar trunk lymph collected from the uninjured side contained much LDH. It can be assumed, that this was a consequence of a contamination with lymph from the injured side throughout cross anatomoses connecting the two trunks. Tissue fluid and lymphatic GOT activities in the burned and control legs showed a pattern similar to that of LDH, only GOT activity did not rise after injury so excessively as LDH.

In the burned extermities both tissue fluid and lymphatic protein concentrations were significantly higher than in the normals and there was no significant difference between the protein content of tissue fluid, crural and lumbar trunk lymph. In the uninjured limb of the same animals tissue fluid total protein concentration was significantly lower than in normal animals. The concentrations in leg and lumbar trunk lymph were about the same as in the normals. Actually in the uninjured extremity of the burned animals no significant difference could be observed between tissue fluid and lymph protein concentrations.

Between the total protein concentrations of tissue fluid and of leg lymph the relationship was linear, the coefficient of correlation was low (0.46). All but one data from the intact extremity of burned animals fell below the regression line (Fig. 7). However, in a similar study made in over 30 normal rabbits the correlation between tissue fluid and lymph protein concentrations was not much better ($r = 0.52$).

The relationship between tissue fluid and prenodal lymph LDH activities in non linear, the best fit, with a high coefficient of correlation ($r = 0.86$) was obtained with a logarithmic regression curve (Fig. 8).

These studies have confirmed that intracellular enzymes are continously released into the tissue fluid. It was also shown, that both in normal and in pathological conditions the enzyme concentrations are markedly higher in subcutaneous tissue fluid than in the lymph originating from the same region. The relationship between tissue fluid and lymph is expressed by a logarithmic regression equation. From this equation follows, that if tissue fluid LDH is doubled, e.g. from 5000 U/ml to 10000 U/ml, lymphatic LDH increases only by 160 units.

Studies in dogs and rabbits have shown that subcutaneous fluid contains significantly more protein than regional lymph. On the other hand no significant difference was observed in the equilibration of intravenously injected radioactive labelled albumin between tissue fluid and lymph (Fig. 9). It follows, that there are important differences in the lymphatic transport of protein molecules according to their origin. The enzymes released from the cells are considerably delayed in their passage accross the interstitial tissue. The labelled protein leaving the blood capillaries is almost immediately taken up by the lymph vessels. These observations suggest a complex structure of the tissue fluid, the presence of at least two compartments. The first compartment is connecting the blood capillaries and the lymphatics and forms the pathway taken be the fluid and protein leaving the capillaries The second is a pool not directly drained by the lymphatics. In the isovolumetric state of the extremity the volume and composition of the first compartment 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 released from the cells and it is a dynamic equilibrium with the first compartment.

The above hypothesis is supported by some other observations made in the present investigations. In the model here outlined the changes occuring in some special situations in the composition of tissue fluid and lymph can be predicted. E.g. if fluid and protein leakage from the capillaries increases, it can be expected that the composition of the second, stationary compartment of the tissue fluid approaches that of the capillary filtrate and lymph. This is what actually happens in the burned extremity where the permeability of the blood vessels to protein is markedly increased, fluid leakage from the damaged capillaries and consequently lymph flow is high and 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. On the other hand 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 tissue. This is reflected by the low protein content of tissue fluid in the control, unburned leg of the animals. The mechanism of the mobilization of the protein pool in the undamaged tissues is not entirely clear. The analysis of hydraulic and oncotic forces would be of great importance. However, it can be expected, that in this situation the stationary compartment of the tissue fluid equilibrates with the mobile part, the "capillary filtrate", and the lymph. In the present experiments it was actually observed that 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 protein 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 therefore changes in the composition of both compartments are reflected in the lymph, which is practically identical with the first compartment of the tissue fluid.

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Discussion

Lassen: The problem of trauma of blood capillaries is always a difficult one. In our sutidies in man a thin needle is inserted subcutaneously and 0.1 ml injected slowly. And that suffices to give some leak of albumin out (as found by *Aukland*) and also a small (0.02%) early arrival in blood. We do in our studies see evidence thereof. Extrapolating it to the state of no trauma, we conclude that in the intact state albumin molecules will not – not even 0.2% of them – pass back through the capillary wall.

One point more: In the plasma-lymph studies reported here you speak of equilibrium. This term is not acceptable in my opinion. Early the specific activity of albumin of plasma exceeds that of the interstitium. At long time the opposite is seen. The two curves only cross once. Only if one would artificially – by many small injections – keep the plasma specific activity constant for some weeks will the system equilibrate. In small animals a shorter time will suffice. But the necessity of keeping the plasma spedific activity constant is the same. Only for the native non-labelled proteins this problem vanishes. Nevertheless also here grave problems of steady-state exist.

Szabo: The term equilibration may be not entirely correct. We use it in lack of a better one.

Well, now for back diffusion. The basic question is wether filtration or diffusion is the main mechanism for protein leakage. If there is an important diffusion of plasma proteins from the capillaries, there must be also a diffusion in the opposite direction. In the case of plasma proteins there is an outward directed concentration gradient and the net result of the exchange process will be protein leakage from the capillaries into the tissue fluid.

In the case of enzymes released from the tissue cells or of labelled protein injected into the tissue there is an oppositely directed concentration gradient. In the latter case initially you have a zero plasma concentration. Accordingly the label flux will be directed from the tissue into the capillaries and there will be no diffusion from the capillaries. The back diffusion of proteins is quite a rapid process, especially in tissues with highly permeable capillaries. This holds both for small and big animals. Of course there are some species differences. The main point is, that from some tissues the major part of the introduced label will disappear in a couple of hours, in others, e.g. in inactive muscle, it takes somewhat longer, but we have both direct and indirect evidence, that the protein from the tissues is not carried away exclusively by the lymphatics and that some part, sometimes the major part of it, gains access directly into the capillaries and veins. This process takes part in the exchange of proteins, accordingly also in the exchange of enzymes between tissue fluid and blood plasma.

Haljamäe: I would like to comment on your statement on a diffusional delay in the interstitial tissue. Our results on interstitial dynamics during hypovolemic shock conditions indicate that there are changes in the degree of hydration as well as in the state of aggregation of the ground substance. Tissues hydration is decreased and the density of the interstitial substance and thereby the density of the colloidal charge is increased. This favours the development of "hidden"

tissue changes, i.e. local in blood not revealable changes. Products from anoxic cells leak into the interstitium but are not cleared from there due to changes in diffusional transport as well as in tissue perfusion. Therefore local immobilization and binding of potentially toxic metabolites occur during shock and thereby potentially toxic effects on vital organs such as CNS or heart are prevented. The diffusional delay in the interstitium is obvious not only on tissue perfusion but also on the state of aggregation of the interstitial colloids i.e. interstitial hydration.