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# Electrolyte Concentrations in Subcutaneous Tissue Fluid and Lymph

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**Summary**

The electrolyte concentrations of subcatenous lymph and tissue fluid collected with the wick technique were compared with those of blood serum. The $Na^+$ and $K^+$ concentrations were found to be higher in tissue fluid and lymph than in serum, $Cl^-$ concentration was lower in lymph, but in tissue fluid it was about equal to the concentration in blood serum. These relationships do not correspond to the Gibbs-Donnan equilibrium. On the basis of electrolyte concentrations and the equilibration of intravenously injected $^{131}I$ albumin it is concluded that the analysis of regional lymph gives reliable information about the steady state concentration of electrolytes and small molecules in the interstitial fluid. Acute changes, especially those of macromolecule concentration in tissue fluid can be more readily followed by the analysis of the fluid obtained by the wick technique.

## *Introduction*

One of the basic assumptions in fluid and electrolyte metabolism has been that since blood plasma and interstitial fluid are separated by a semipermeable membrane the relationship between their electrolyte concentrations corresponds to the values predicted by the *Gibbs-Donnan* equilibrium. Actually no direct methods for sampling and analysis of normal tissue fluid were available (7). Recently, however, several methods became available for the collection of interstitial fluid from various tissues of experimental animals. *Szabó* et al. (10, 14) have compared two of these, the perforated capsule method (3) and the wick technique (2) and on the basis of the equality in the protein concentration and composition of the samples obtained by the two methods both in dogs and rabbits they have concluded that the composition of the samples reflects that of the interstitial fluid. Later a "liquid paraffin cavity" method has been developed for the sampling of nanoliter quantities of subcutaneous tissue fluid (4) and the electrolyte and protein concentrations of this fluid were analyzed and compared to those of blood plasma and of the fluid obtained by the capsule method (1). It was observed, that the electrolyte composition of the subcutaneous tissue fluid samples especially of those obtained by the paraffin cavity method, does not correspond to that of a plasma ultrafiltrate, i.e. to the composition expected on the basis of the *Gibbs-Donnan* equilibrium. The $Na^+$ and $K^+$ concentrations were actually higher and $Cl^-$ concentration lower in the protein poor interstitial fluid than in blood plasma.

The problem of transcapillary water and electrolyte exchange and equilibrium and in connection with it that of lymph formation and composition being of no mean interest, the composition of subcutaneous tissue fluid collected by the cotton wick technique and of regional lymph is compared in the present study with that of blood serum.

## *Materials and Methods*

The experiments were done in male rabbits with a body weight of 2.8 to 3.8 kg in pentobarbital anaesthesia (30 mg/kg body weight by intravenous injection). A prenodal popliteal lymph vessel, carrying predominantly cutaneous lymph was cannulated. Lymph collection was immediately started and it was continued for 1 h. Cotton threads, 5 to 6 in number and 3 to 4 cm long, previously

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**Table 1** Electrolyte content of biological fluids (X ± S.D.; n = 15)

| | | $Na^+$ | $K^+$ | $Cl^-$ | Protein g/liter |
|---|---|---|---|---|---|
| *Serum* | mmol/liter | 138 ± 3 | 3.38 ± 0.40 | 105 ± 6 | 48.8 ± 10,3 |
| | mmol/kg water | 145 | 3.54 | 110 | |
| *Tissue fl.* | mmol/liter | 153 ±9$^+$ | 3.68 ± 0.50 | 106 ± 6 | 23.2 ± 6.8 |
| | mmol/kg water | 156 | 3.56 | 108 | |
| *Lymph* | mmol/liter | 145 ±9$^+$ | 3.70 ± 0.72$^+$ | 99 ± 7$^{x+}$ | 21.1 ± 8.6 |
| | mmol/kg water | 148 | 3.78 | 101 | |

$^+$ significant difference compared to blood serum ($p < 0.05$)
$^x$ significant difference between lymph and tissue fluid ($p < 0.05$)

soaked overnight in about half of the experiments in physiological saline, in the rest of the cases in Ringer-lactate solution (containing 140 mmol/liter $Na^+$, 106 mmol/liter $Cl^-$ and 4 mmol/liter $K^+$) were sewn into the subcutaneous tissue of the shank at the beginning of lymph collection and withdrawn at its end, i.e. after 1 h. It was found that the composition of the bath fluid does not influence significantly the results. Accordingly the data obtained by the two methods are summarized in a single table.

In previous investigations it was established that evaporation can lead to a considerable increase of solute concentration in lymph and especially in tissue fluid. To avoid water loss from the protruding ends of the cotton threads immediately after their introduction the leg of the animals was wrapped in a sheat of plastic and lymph was collected in small vials under mineral oil. When pulled out the cotton threads were placed in a small syringe and the fluid was expressed manually into similar containers as used for lymph collection.

In other two groups of animals $^{131}I$ labelled human serum albumin was injected intravenously and blood samples were withdrawn at half hourly intervals. In the first group of 6 rabbits tissue fluid and lymph were collected between the 1st and the 2nd hour after the administration of the tracer and in the second group of 8 animals between the 5rd and 4th hour.

The sodium and potassium concentrations in the samples were determined by flame photometry, the chloride concentrations with a silver precipitation technique, total protein with the Folin reagent according to *Lowry* et al. (5) and the albumin fraction by zone electrophoresis in agarose gel. The radioactivities were measured in a well type Na/Tl scintillation counter. The results in the text, table and figures are means with ± S.D.

*Results*

In the 15 rabbits $Na^+$ concentration in blood serum was 138 mmol/liter, in tissue fluid 153 mmol/l and in the regional lymph 145 mmol/l. The differences between plasma and tissue fluid or lymph concentrations were significant (paired $t$ test $p < 0.05$). Potassium concentration was also higher in lymph and tissue fluid than in serum. On the other hand chloride concentration was lower in lymph (99 mmol/l) and (not significantly) higher in tissue fluid (106 mmol/l) than in blood serum (105 mmol/l) (Table 1).

The electrolyte concentrations should be, however, compared on the basis of their concentration in the water phase of the body fluids. The values computed per kg water are therefore also shown in Table 1. This makes, however, only little difference in the final conclusion: the cation concentrations remain higher in lymph and tissue fluid than in blood serum, and on the other hand chloride concentration is sensibly lower in lymph water than in serum water, in tissue fluid water it is equal to the concentration observed in serum water. The total protein concentration in the interstitial fluid was 47.5 % of plasma concentration and that of lymph 43.4 %.

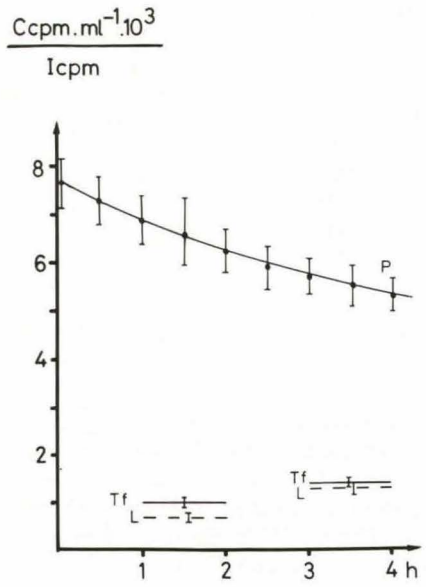


Fig. 1 Concentrations of labelled albumin (protein bound radioactivity in the biological fluids per injected activity) in the leg lymph (L), subcutaneous tissue fluid (TF) and blood plasma (P)

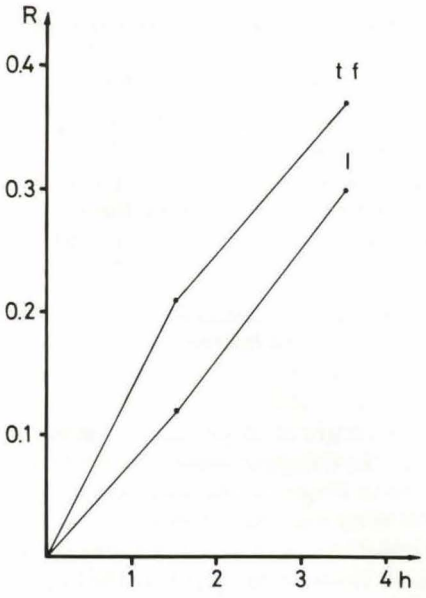


Fig. 2 Lymph per plasma (l) and tissue fluid per plasma (tf) specific activity ratios

In the second hour after its injection the lymphatic concentration of labelled albumin was 6.6 % of plasma concentration and the concentration in tissue fluid 9.8 %. In the next period, between the 3rd and 4th hour the relative concentrations rose to 13.4 and 14.1 % respectively (Fig. 1). The lymph or tissue fluid to plasma activity ratios per gram albumin were in the first period 0.12 (lymph) and 0.23 (tissue fluid) and in the second period 0.30 and 0.37 respectively (Fig. 2). The differences between the lymphatic and interstitial fluid activities and concentration ratios were significant in the first period, between the 1st and 2nd hour after the injection of the tracer.

*Discussion*

In previous studies the opinion was expressed that the fluids obtained by the implanted capsule and wick methods are true samples of the interstitial fluid (11, 14). This conclusion was based on the equality of their protein concentrations. These studies were made, however, in a steady state where no acute changes were expected. Consequently dynamic studies were made with enzymes escaping from the tissue cells and with intravenously injected labelled albumin (12, 13, 14). These experiments were done with the wick technique because it was assumed that the large fluid pool inside the capsule is separated from the surrounding tissue by the capsule lining formed of fibrous connective tissue. Accordingly, concentration changes inside the capsule would follow only sluggishly those occuring in the free interstitial fluid. It could be shown that the activities of enzymes escaping from the cells rise in the subcutaneous wick fluid more rapidly and to a much higher level than in regional lymph (12, 13) and the intravenously injected labelled protein attains after 24 h in lymph and wick fluid similar concentrations (14).

In the present investigations it was observed that both $Na^+$ and $Cl^-$ concentrations are a little higher in tissue fluid than in lymph. It may be assumed, however, that these minor differences are due to methodological reasons

mainly to the evaporation of water from the cotton threads during the manipulation.

The concentration of injected $^{131}$I-albumin rises, especially in the first hours after injection more rapidly in tissue fluid than in lymph. The delay may be due to the diffusion of the large protein molecules across the interstitial matrix and lymph capillary wall and to the dead space represented by the collecting lymphatics and the cannula. On the other hand it seems obvious, that in steady state the electrolyte concentration of lymph closely reflects that of tissue fluid. Accordingly regional lymph samples seem to be more suited for the study of steady state concentrations of electrolyte and small molecules in the interstitial fluid, but from samples obtained by the wick technique better information can be gained about the concentration of macromolecules and about acute changes in the composition of tissue fluid.

The electrolyte data in the present study, similarly to those observed by *Haljamäe* and *Freden* (4) do not correspond to those predicted by the Gibbs-Donnan equilibrium. One should expect in tissue fluid water $Na^+$ and $K^+$ concentrations about 2 to 3% lower, and $Cl^-$ concentration by the same percentage higher than their respective concentrations in plasma water. Actually $Na^+$ concentration is by 2%, $K^+$ concentration by 7% higher and $Cl^-$ concentration by 7% lower in lymph water than in serum water. This discrepancy can be explained by the presence in the tissue fluid of anions absent or present only in much lower concentration in blood serum. These anions must be poorly diffusible macromolecules which cannot cross the capillary barrier or they may be fixed to the connective tissue matrix. On the other hand it was shown already by *Schade* and *Menschel* (8) that connective tissue takes up water in solutions isotonic with blood plasma. Rat corium swells in physiological saline solution (6) and this is but little influenced by the presence of serum protein in the bath fluid (9). The hyperosmolality which is due to the presence of fixed ions may be the cause of the negative hydrostatic pressure observed in subcutaneous interstitium. As the nature of the fixed ions, *Amundson* and *Haljamäe* (1) have suggested that they may be the negatively charged carboxyl and sulfate groups of the glucosaminoglycans of the ground substance, but the imbalance of electrolyte concentrations may be due also to diffusible negatively charged products of cell metabolism present in higher concentration in the interstitial fluid than in blood serum.

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