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Composition of Interstitial Fluid

Niels Fogh-Andersen,[1,4] Burton M. Altura,[2] Bella T. Altura,[2,3] and Ole Siggaard-Andersen[1]

In several previous experiments to determine the composition of interstitial fluid, the results varied depending on the collecting technique, and the electrolyte concentrations differed from those of a hypothetical ultrafiltrate of plasma. In our approach, since a change of position from standing to supine is accompanied by hemodilution with interstitial fluid, we used the changes in hematocrit and composition of plasma in 20 subjects before and after lying down to calculate the composition of added interstitial fluid. The estimated protein concentration was 20.6 g/L, and the concentrations of total calcium and magnesium were low, in accord with a lower concentration of protein-bound calcium and magnesium. The activity of free cations was also lower, in agreement with a Donnan equilibrium potential of 1 mV across the endothelium. The concentration of leukocytes and platelets decreased according to the hemodilution, implying no escape or mobilization of these elements.

Indexing Terms: *electrolytes/hematocrit/hemodilution/ion-selective electrodes/ionized calcium/ionized magnesium/plasma volume/sample handling*

No direct methods for sampling interstitial fluid are currently available. The composition of interstitial fluid, which constitutes the environment of the cells and is regulated by body homeostasis, has previously been measured by the suction blister or liquid paraffin techniques or by implantation of a perforated capsule or wick. The results have varied, depending on the sampling technique and animal species investigated. In one study, the ion distribution between vascular and interstitial compartments agreed with the Donnan equilibrium (*1*); in others, the concentrations of sodium and potassium were higher in interstitial fluid than in plasma (*2*, *3*). The concentration of protein in interstitial fluid is lower than in plasma, and the free ion activities theoretically differ from those of plasma because of the Donnan effect. In spite of these differences, and for practical reasons only, plasma is used clinically to monitor fluid and electrolytes. The relation between plasma and interstitial fluid is important in treating patients with abnormal plasma volume or homeostasis.

We measured hematocrit (Hct) and various analytes in the plasma of 20 subjects before and after a physiological hemodilution during a change in posture to determine the composition of interstitial fluid.[5]

Materials and Methods

The population consisted of 20 healthy volunteers, 16 women and 4 men, mean age 30 years (range 20–46 years), from the Department of Clinical Chemistry at Herlev Hospital. The study (KA 94264) was approved by the local ethical committee.

Blood samples taken (without venostasis by N.F.-A.) from an arm vein of each subject (*a*) while standing and (*b*) after 10 min supine were placed in three Vacutainer tubes (Becton Dickinson, Rutherford, NJ). The desired hemodilution was obtained within 10 min without major regulatory changes. One tube was stabilized with K_3EDTA for hematology, and the other two were plain tubes for serum. Serum for determination of ionized magnesium, sodium, and potassium was separated and frozen for analysis the following week at the State University of New York Health Science Center at Brooklyn. Extra CO_2 was added before freezing to yield a pH of 7.33 ± 0.08 (mean ± SD) at the time of measurement. A previous study (*4*) reported no detectable effect on the ionized magnesium value of repeated freeze–thaw cycles. The other analytes were measured immediately at Herlev Hospital.

Ionized magnesium adjusted to pH 7.4 was measured on a Stat Profile 8 from NOVA Biomedical (Waltham, MA) as previously described (*5*). The instrument also measured ionized calcium, pH, sodium, and potassium values by direct potentiometry with ion-selective electrodes (ISEs). pH, actual ionized calcium, and ionized calcium adjusted to pH 7.4 were measured on anaerobically treated samples with an ICA2 from Radiometer (Copenhagen, Denmark). Total magnesium concentration was measured on an RA XT (Bayer, Tarrytown, NY). Hct, erythrocytes and erythrocyte indices [mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC)], hemoglobin, leukocytes, and platelets in blood were measured on an H*2 (Bayer). Each individual's concentration of platelets and leukocytes in blood was converted to the concentration in plasma by division with (1 − Hct) to estimate the concentration of platelets and leukocytes in exchanged interstitial fluid by the same formula as used for the other analytes. The concentrations of total protein, albumin, total sodium, potassium, calcium, CO_2, and phosphate were measured with a SMAC 3 (Bayer). The SMAC 3 involves indirect

[1] Department of Clinical Chemistry, Herlev Hospital, DK 2730 Herlev, Denmark.

Departments of [2] Medicine and [3] Physiology, SUNY Health Science Center at Brooklyn, 450 Clarkson Ave., Brooklyn, NY 11203.

[4] Author for correspondence. Fax (45)44535332; e-mail nfa@biobase.dk.

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[5] Nonstandard abbreviations: Hct, hematocrit; ISE, ion-selective electrode; MCV, mean corpuscular volume; and MCHC, mean corpuscular hemoglobin concentration.

potentiometry with ISEs to measure the total concentrations of sodium and potassium. Because the samples are diluted before measurement, the concentrations of protein, lipid, water, or complexing anions, and the ionic background do not affect the accuracy of the total concentrations obtained by the SMAC 3. Sodium and potassium concentrations obtained by direct potentiometry are proportional to the activity. Mean values obtained by the two methods are identical in normal persons, but some results may differ because the concentrations of water and ions may vary.

The general term plasma here refers to the circulating plasma and all the forms in which it is sampled.

Calculations

The volumes of blood and plasma and the Hct before the change in posture were BV1, PV1, and Hct1. After the subjects were recumbent for 10 min, the values were BV2, PV2, and Hct2. Assuming that the Hct of peripheral blood equalled the Hct of the whole body, and that the total volume of circulating erythrocytes did not change, then

$$PV1 = BV1(1 - Hct1)$$

$$\text{and} \quad PV2 = BV2(1 - Hct2) \qquad (1)$$

and

$$BV1 \cdot Hct1 = BV2 \cdot Hct2 \qquad (2)$$

Dividing Eq. 2 by Hct2 yields

$$BV1(Hct1/Hct2) = BV2 \qquad (3)$$

Substituting Eq. 3 into Eq. 1 yields

$$PV2 = BV1(Hct1/Hct2)(1 - Hct2) \qquad (4)$$

The added amount of substance x (n_x) was

$$n_x = PV2 \cdot c_x2 - PV1 \cdot c_x1$$

$$= BV1[(Hct1/Hct2)(1 - Hct2)c_x2 - (1 - Hct1)c_x1] \qquad (5)$$

where c_x1 and c_x2 are the respective plasma concentrations of x. The volume of added interstitial fluid V was

$$V = PV2 - PV1$$

$$= BV1(Hct1/Hct2)(1 - Hct2) - BV1(1 - Hct1)$$

$$= BV1[(Hct1/Hct2) - 1)] \qquad (6)$$

Division and rearrangement yield the following composition of interstitial fluid (IF):

$$c_x(IF) = n_x/V$$

$$= \frac{c_x2(1 - Hct2)Hct1 - c_x1(1 - Hct1)Hct2}{Hct1 - Hct2} \qquad (7)$$

The blood volume BV1 cancels out during division. In this way the composition of the exchanged interstitial fluid can be determined from the changes in Hct and concentration only. We used the mean Hct and plasma concentrations of the subjects to avoid division by small, relatively imprecise individual differences in Hct and thus obtained as accurate a composition of interstitial fluid as possible by using Eq. 7. The change in Hct was subject to analytical variation, and division by individual values of (Hct1 − Hct2) yielded unlikely results if the denominator was close to zero.

The relative dilution of plasma by interstitial fluid was expressed by van Beaumont's formula (*6*):

$$(PV2 - PV1)/PV1$$

$$= (Hct1 - Hct2)/[(1 - Hct1)Hct2] \qquad (8)$$

Ionized calcium and magnesium in plasma are buffered by the proteins, with the respective buffer values of $dctCa/dcCa^{2+}$ and $dctMg/dcMg^{2+}$ being proportional to the protein concentration. The buffer values express the ratio between changes in total and free ion concentrations after addition or removal of the ion. Interstitial fluid with its lower protein concentration has a lower buffer value than plasma. The buffering of ionized calcium and magnesium may diminish the change in free ion concentration in plasma and therefore underestimate the calculated difference between interstitial fluid and plasma according to Eq. 7, depending on the calcium and magnesium buffer values of interstitial fluid and plasma. As a first approximation we assumed equal buffer values, but we shall later discuss the importance of buffer values and pH.

Statistical Methods

The statistical significance of changes was evaluated by the paired t-test, and that of correlations was evaluated by the linear regression model. Probabilities are reported as not significant, $P < 0.05$, $P < 0.01$, or $P < 0.001$.

Results

The mean Hct decreased from 0.4023 to 0.3785 after 10 min in the supine position, corresponding to an increase in plasma volume of 10.5%. In a normal man with initial blood and plasma volumes of 5 and 3 L, respectively, the added volume of fluid would be 315 mL.

Table 1 shows the mean hematology results, and Table 2 shows the mean concentration of various analytes in plasma and their concentration in interstitial fluid according to Eq. 7. Hct decreased relatively more

Table 1. Mean hematology results for 20 subjects during standing and after 10 min supine.

	Standing	Supine	Interstitial fluid
Hematocrit	0.4023	0.3785[a]	
B-Hemoglobin (mmol/L)	8.37	7.93[a]	
B-Platelets (10^9/L)	247	233[a]	
P-Platelets (10^9/L)	412	373[a]	3
B-Leukocytes (10^9/L)	7.12	6.70[a]	
P-Leukocytes (10^9/L)	11.97	10.84[a]	0.09

[a] $P < 0.001$ by paired t-test.

The concentration in interstitial fluid was determined with Eq. 7. B, blood; P, plasma.

than hemoglobin, 5.9% vs 5.3%, but the difference was not statistically significant ($P = 0.19$). MCV decreased 0.8 fL ($P < 0.001$). No significant change occurred in MCHC. Part of the decrease in Hct may be the result of the change in pH and P_{CO_2}, which would affect MCV and the distribution of solutes and water across the erythrocyte membrane.

The calculated platelet and leukocyte concentrations in interstitial fluid were close to zero, implying no cellular escape or mobilization during the change in posture. It also implies a certain degree of accuracy of the method, because zero was the most likely result.

Potassium and phosphate decreased more than could be explained by hemodilution, lower protein concentration, or Donnan equilibrium only. The decrease in serum potassium may be related to the lower concentration of platelets after the change in posture. Extrapolation to zero platelet concentration yielded a total potassium of 3.12 mmol/L and an ionized potassium concentration of 3.99 mmol/L by direct ISE.

The decrease in phosphate may be a result of the increase in pH. A strong negative correlation existed between phosphate and pH independently of the change in posture ($r = -0.61$ before and -0.60 after, respectively, $P < 0.001$).

The different concentration of free cations in interstitial fluid and plasma corresponded to a Donnan equilibrium potential (interstitial fluid positive) by the Nernst equation of 0.8 mV for Ca^{2+}, 0.7 mV for Mg^{2+}, 1.2 mV for Na^+, and 2.78 mV for K^+. The Donnan potential is a consequence of electroneutrality and equal electrochemical potentials of the diffusible ions at equilibrium. According to the Nernst equation, the electric potential difference (= electric work per unit of ion) must equal the difference in chemical potential (= chemical work per unit of ion), or

$$zF(E_1 - E_2) = RT \ln(a_1/a_2)$$

where z is the charge number, $F = 96484.56$ C/mol, E is electric potential, $r = 8.31441$ J/(mol · K), T is absolute temperature, a is activity, and 1 and 2 denote two solutions separated by a membrane.

Table 2. Mean plasma concentrations in 20 subjects during standing and after 10 min recumbent, and estimated concentration in interstitial fluid (mmol/L).

	Standing	Supine	Interstitial fluid
Total protein (g/L)	73.7	68.6[a]	20.6
pH	7.358	7.383[a]	
Albumin	0.676	0.630[a]	0.188
Total calcium	2.365	2.288[a]	1.551
Ca^{2+} (pH 7.4)	1.257	1.250[b]	1.183
Total magnesium	0.887	0.866[a]	0.666
Mg^{2+} (pH 7.4)	0.532	0.530	0.506
Total sodium	138.8	138.4	134.6
Sodium (direct ISE)	141.8	141.2[a]	135.7
Total potassium	4.28	4.17[c]	3.17
Potassium (direct ISE)	4.41	4.37	3.97
Total CO_2	29.7	29.2[b]	23.9
Phosphate	1.177	1.123[a]	0.610

[a] $P < 0.001$, [b] $P < 0.01$, [c] $P < 0.05$, by paired t-test.

Discussion

Renoe et al. (*7*) reported similar changes in plasma for various analytes during a change in posture, without discussing the mechanism. We argue here that the changes are caused by a hemodilution with interstitial fluid in Donnan equilibrium with plasma, and we used the changes to determine indirectly the composition of interstitial fluid. This method is more refined than previous sampling techniques, which may have influenced the composition of the analyzed interstitial fluid.

The concentration of protein in the exchanged interstitial fluid was 20.6 g/L, which was identical to the concentration in lymph from skin or skeletal muscle (*8*), and surprisingly high. If the capillary endothelium were an effective ultrafilter, the retained fraction of protein would be identically high for passage either way, and the protein concentration in interstitial fluid passing into the blood should be close to zero. Increasing the plasma volume 10.5% by interstitial fluid with zero protein concentration would decrease the protein concentration in plasma by $1/(1 + 0.105) = 9.5\%$. However, total protein and albumin concentrations in plasma decreased only 6.9%, implying identical filtration and distribution ratios for albumin and globulin. The estimated protein concentration in exchanged interstitial fluid was close to the concentration in lymph. The endothelium appeared more like a depth filter with low selectivity towards albumin and globulin than it resembled an effective ultrafilter.

Bent-Hansen (*9*, *10*) suggested two possible distinct barriers to protein: (*a*) the leaky capillary membrane itself, which allows a rapid bilateral diffusive transport of albumin between plasma and a compartment below the endothelium; and (*b*) a barrier formed by the surrounding interstitium with its gel of glucosaminoglycans. When fluid passes from the interstitium to plasma, it will flush the subendothelial compartment and move albumin along.

Results of our study (*11*) of blood donors during donation of 450 mL of blood were similar to those of this study. Blood samples were obtained from the donors immediately before and after the blood donation, which lasted 10 min. The calculated concentration of albumin in the donors' interstitial fluid was 0.187 mmol/L, almost identical to the present value of 0.188 mmol/L. This favors the depth-filter theory, because a subendothelial compartment probably would not yield the same concentration in two such different situations.

Part of the decrease in potassium may have been caused by the lower platelet concentration after the change in posture. Potassium is liberated from the platelets during coagulation (*12*), which explains the difference between plasma and serum potassium, and we used serum. Ladenson et al. (*13*) observed a 0.34 mmol/L lower potassium concentration in plasma than in serum, in agreement with our value after extrapo-

lation to zero platelet concentration. The rest of the difference between interstitial fluid and plasma agrees with the Donnan theory.

The decrease of 0.054 mmol/L in phosphate concentration indicated a phosphate concentration in interstitial fluid of only 0.61 mmol/L. The decrease was probably caused by an increase in pH. Siggaard-Andersen (*14*) reported a change in plasma phosphate of −2 mmol/L per pH unit, in good agreement with our observed value of −2.16 mmol/L per pH unit. The mechanism for pH dependence was probably an increase in the concentration of organic phosphates in the erythrocytes with increasing pH (*14*). In plasma, 20% of phosphate is protein bound. Part of the decrease in plasma phosphate could be explained by a lower concentration of protein-bound phosphate in interstitial fluid. The Donnan distribution and the higher concentration of water in interstitial fluid would act in the opposite direction and increase the phosphate concentration in interstitial fluid relative to that of plasma.

The increase in pH in recumbent subjects might contribute to the observed changes by affecting the distribution of water. An increase in pH of 0.025 would cause 4.5 mL of water to shift from erythrocytes to plasma, diminishing Hct by ~0.001 (*15*). The effect on analytes confined to plasma would be −0.15%, which is not negligible in the present context. However, most of the water probably had diffused into the tissues after 10 min.

Calculation of the composition of interstitial fluid required that no net diffusion occur between interstitial fluid and plasma. Electrochemical disequilibrium is a prerequisite for net diffusion between interstitial fluid and plasma. The increase in pH reduced the actual ionized calcium concentration in plasma by 0.02 mmol/L. We did not measure actual ionized magnesium at the original pH of plasma. However, because the effect of pH on ionized magnesium is about one-third of the effect on ionized calcium, and because the concentration of ionized magnesium was lower than of ionized calcium, the pH decreased ionized magnesium concentration in plasma by <0.01 mmol/L. The concentration of ionized calcium and magnesium in interstitial fluid must also be decreased by the increasing pH, but less than in plasma because the protein concentration in interstitial fluid was lower. Some net calcium and magnesium may therefore have diffused from the tissues into plasma. Furthermore, the plasma proteins acted as buffers and released protein-bound calcium and magnesium when interstitial fluid was added, diminishing the effect of the added interstitial fluid on ionized calcium and magnesium in plasma. Both effects may overestimate the concentration of ionized calcium and magnesium in interstitial fluid.

The difference in protein concentration between plasma and interstitial fluid corresponds to a difference in protein net charge of 12 mmol/L. Together with a salt concentration of 150 mmol/L, this yields a calculated transendothelial Donnan potential of 1.07 mV, interstitial fluid positive (*16*). In this study, calcium and magnesium had slightly lower and sodium and potassium had higher Nernstian distribution potentials than 1.07 mV, but the discrepancy has already been accounted for. Classical physical chemistry can explain the observed difference between interstitial fluid and plasma, in agreement with Gilanyi et al. (*1*).

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