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THE ERYTHROCYTE EFFECT ON THE LIQUID JUNCTION POTENTIAL: AN OSMOTIC DILUTION PHENOMENON

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ABSTRACT

The liquid-junction potential of the junction 'saturated KCl || whole blood' is about 0.6 mV higher than of the junction 'sat. KCl || plasma'. The difference increases to about 1.4 mV for a hematocrit of 75 %. Replacing KCl with other salts alters the potential difference dramatically from about - 3 mV for $CaCl_2$ (3 mol/l) to +4.8 mV for potassium citrate (1.5 mol/l) (both referring to a hematocrit of 75 %), but the potential differences vanish when the salt solutions are diluted to a physiologic concentration. The effects, we thought, were due to a salting out of proteins at the liquid junction with the formation of an ion-exchange layer, changing the rate of diffusion of the ions from the bridge solution; e.g. K^+ and Cl^- would no longer be equitransferent but K^+ would move faster than Cl^-. Unfortunately there is little evidence for this theory on the basis of known data for the protein binding of ions. We now believe that the effects are due to an osmotic crenation of the red cells causing a dilution of the suspension medium. Firstly, calculations by the Henderson equation with the new theory almost perfectly explain the experimental data, secondly the effect is also observed with washed red cells suspended in a protein free buffer, and thirdly erythrocyte ghosts also change the junction potential.

Key words: electrochemistry, ion-selective electrodes; potentiometry;

It was observed by Severinghaus et al. in 1956 that the pH of whole blood was about 0.008 lower than the pH of the corresponding plasma separated anaerobically at 37 °C [1]. This was confirmed by others and it was shown to be due to an effect of the red cells at the 'saturated KCl||blood' junction, increasing with increasing hematocrit and disappearing with a physiologic concentration of the bridge solution [2-5]. Although the mechanism is obscure it is often referred to as the 'red cell suspension effect' because it is thought to be related to the 'suspension effects' demonstrated in colloidal systems by Jenny et al. [6]. The erythrocytes are large polyanions with many sialic acid residues on the suface, but expressed as a substance concentration of anion in the blood it amounts to only 0.1 mmol/l (for a normal erythrocyte concentration) and it cannot account for the positive junction potential.

The red cell bias became more important with the introduction of ion-selective electrodes for Ca^{2+}, which have only half the sensitivity of electrodes for monovalent ions: ionized calcium is measured erroneously high in whole blood, up to +11 % when the hematocrit is high.

We therefore studied the phenomenon again and found that the effect was strongly dependent on the type of saltbridge solution: with certain salts we found a surprisingly large *negative* bias, with others a large *positive* bias (Figs. 1 and 2). With certain mixed saltbridge solutions such opposite effects would cancel out [7,8]. With sodium formate the bias was minimal and this salt seems to be ideal for potentiometric measurements in whole blood [9].

We previously believed that these dramatic effects of erythrocytes on the liquid junction potential were due to proteins salting out at the liquid junction, forming an ion-exchange layer which would affect the rate of diffusion

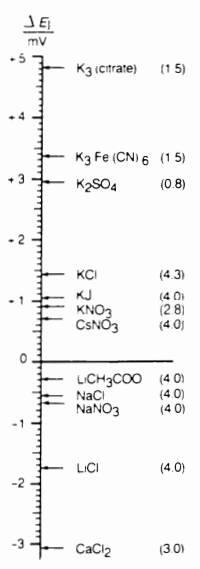

Fig. 1. The bias caused by erythrocytes (volume fraction 0.75) on the liquid-junction potential (ΔE_j) of the junction "Bridge solution || Blood" with 12 different salt bridge solutions. The numbers in parenthesis are substance concentrations in mol/l [7].

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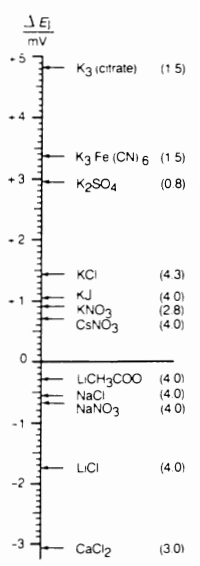

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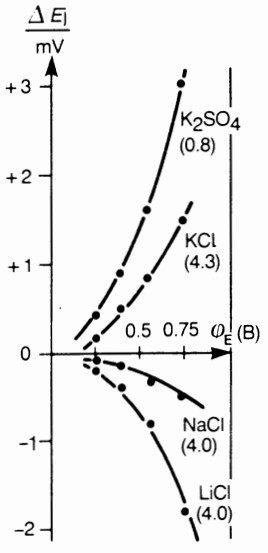

Fig. 2. The effect of erythrocytes on the liquid-junction potential (ΔE_j) as a function of the volume fraction of erythrocytes in the blood (ØEry(B) with different composition of the salt bridge solution [7].

of the ions from the salt bridge solution into the test solution [8]. We had to assume that different ions were slowed down to a different extent by the ion exchange layer, for example that Cl^- was slowed down more than K^+ resulting in a positive junction potential. Unfortunately the effects of the different saltbridge solutions could not be predicted on the basis of known data for the binding of the various ions to proteins.

During the meeting in Stresa it was again postulated that proteins have a large and unpredictable effect on the liquid-junction potential even to the extent of invalidating direct potentiometric measurements in serum, plasma or whole blood for clinical purposes [Payne, Saris, and others]. The erythrocyte effect was taken as an indication of such deleterious protein effects.

On the train on our way home the two of us discussed the problem and we both agreed that red cells do not 'precipitate' on contact with a saturated KCl solution: under the microscope we observe a crenation of the cells, which means that water is extracted by osmotic forces, diluting the surrounding plasma. In our very schematic model, illustrated in Fig. 3, we get one liquid-liquid junction between the

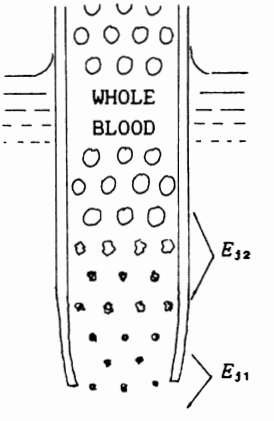

Fig. 3. Schematic magnification of the liquid-liquid junction between a hypertonic bridge solution and whole blood. A layer of diluted plasma with crenated red cells is formed.

concentrated bridge solution and diluted plasma (with suspended dried up cells) and a second liquid-liquid junction between the diluted plasma and the undiluted plasma (with undisturbed red cells).

The purpose of this paper is to test this hypothesis by calculations with the Henderson equation.

MATERIAL AND METHODS

Our new model of the liquid-liquid junctions is the following:

		E_{j1}		E_{j2}	
Blood:	concentrated bridge solution	||	diluted plasma (with crenated erythrocytes)	||	undiluted plasma (with normal erythrocytes)
Plasma:	bridge solution		E_{j3} ||		undiluted plasma

Erythrocyte effect on junction potential: $(E_{j1} + E_{j2}) - E_{j3}$.

The Henderson equation for junction potential of '1||2' is:

$$E_j = (-R \cdot T/F) \cdot [(h_2 - h_1)/(k_2 - k_1)] \cdot \ln(k_2/k_1),$$

$h = \Sigma(c_i \cdot l_i^\circ/z_i)$,
$k = \Sigma(c_i \cdot l_i^\circ)$: conductivity of the solution,
c_i : concentration of the ions,
z_i : charge number of the ions,
l_i° : limiting molar conductance of i, (listed in Table 1).

The calculations were performed for the same bridge solutions that we have previously used experimentally (see Fig. 1). For normal plasma we only took Na^+, Cl^- and HCO_3^- into account with concentrations of 0.150, 0.100 and 0.025 mol/kg, respectively. If all the water from the red cells dilutes the plasma and the hematocrit is 75% we would get a dilution of the plasma concentrations to about 33%. However, using this dilution effect in the calculations overestimated the red cell effect and the best fit to the experimental data was obtained with a dilution to 66%.

Table 1. Limiting molar conductance of ions at 37 °C. Unit: mS·m²·mol⁻¹ [9,10].

H^+	40.63	OH^-	23.9	H_2PO_4	4.8
Rb^+	9.6	CN^-	10.4	Tes^-	3.56
Cs^+	9.54	Br^-	9.73	Bes^-	3.44
K^+	9.13	Cl^-	9.55		
NH_4^+	9.1	J^-	9.5		
$CH_3NH_3^+$	7.7	NO_3	8.7		
$Imidazol^+$	6.55	CNO^-	8.4		
Na^+	6.40	$HCOO^-$	6.79		
Li^+	5.02	HCO_3	5.86		
$Tris^+$	3.84	CH_3COO^-	5.3		
Ca^{2+}	15.24	SO_4	20.1	$Fe(CN)_6$	39.0
Mg^{2+}	13.8	$(COO)_2$	18.2	$Citrate^{3-}$	26.6
		HPO_4	15.12		

RESULTS AND DISCUSSION

The correlation between the calculated and the measured data is remarkably good (Fig. 4). The correlation coefficient is 0.97, hence 95 % of the variation is explained by the model, and only 5 % remains as residual variation. There is no need to postulate any special protein effect.

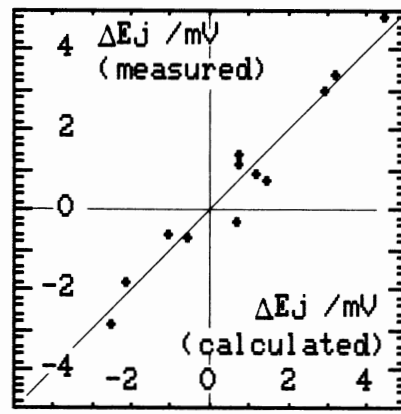

Fig. 4. The bias caused by erythrocytes (volume fraction 0.75) on the liquid-junction potential (ΔE_j) of the junction "Bridge solutions || Blood" with different salt bridge solutions. The abscissa shows the values calculated on the basis of the present osmotic dilution theory. The ordinate is identical with the scale shown in Fig. 1 which shows the previously published directly measured values [7]. Linear regression: $y = 1.02 \cdot x - 0.05$, $r = 0.974$.

To confirm the theory we have performed a few preliminary measurements with washed erythrocytes suspended in a protein free buffer. Such artificial blood with protein free 'plasma' exhibits the same erythrocyte effects on the liquid junction potential as

normal whole blood. The same is the case with hemoglobin free erythrocyte ghosts. Finally the observed effects can be reproduced with normal plasma by interposing a small layer of diluted plasma between the concentrated bridge solution and the plasma. Details of these new measurements will be published separately.

Our calculations and new experimental data strongly indicate that the old 'red cell suspension effect' is neither a classical suspension effect (due to large immobile ions) nor a special protein effect (due to an ion-exchange layer) but rather a simple osmotic dilution phenomenon.

We believe that the protein effects on the liquid junction potential with a saturated KCl bridge solution are small [11] and that the effects which have been ascribed to protein errors on the liquid junction potential [12] are in fact simple Donnan phenomena [13-15].

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