BBA 71063

Chloride and sodium permeabilities of human red cells

Both the exchange of chloride isotopes during steady state distribution of non-radioactive chloride[1] and the net exchange of chloride for bicarbonate across the human red cell membrane[2] occur rapidly at room temperature. The rate constant of chloride exchange is 3.1 sec^{-1} at 23° meaning that the exchange is 50 % completed in 0.2 sec (ref. 1). The net exchange of chloride for bicarbonate has reached equilibrium within one second at 23° and a Q_{10} of 1.5 has been reported between 0 and 24° (ref. 2). Assuming that both net exchange and self-exchange of chloride are affected similarly by temperature, one might, therefore, by extrapolation conclude that exchanges of chloride reach half equilibrium values in 0.5 sec at 0°. Nevertheless, we have observed that the rate of chloride exchange is slowed drastically by a lowering of temperature to permit a detailed study between 0 and 10° by a modification of the erythrocyte filtration technique of MAWE AND HEMPLING[3]. We studied the exchange of $^{36}Cl^-$ between human red cells and a medium with the electrolyte composition of normal plasma (*cf.* Fig. 1). The rate constant of chloride exchange at 0° and pH 7.40 was 0.039 sec^{-1} (range, 0.037–0.042; S.D., 0.002; n = 5). This means that the half time of chloride exchange is 18 sec at 0°. When the temperature is increased from 0 to 10° the rate of chloride exchange increases by a factor of 7 from 0.04 to 0.3 sec^{-1}. Results obtained at intermediate temperatures appear in Fig. 2. 80–90 % equilibration of $^{36}Cl^-$ between cells and medium was reached within 2 sec at 18°. An estimate of the rate constant is 1 sec^{-1}, the same order of magnitude as found in the two cited studies[1,2].

Fig. 2 shows that the logarithm of the rate constant bears a linear relation to the reciprocal absolute temperature, and an apparent activation energy of 33.2 (S.D.,

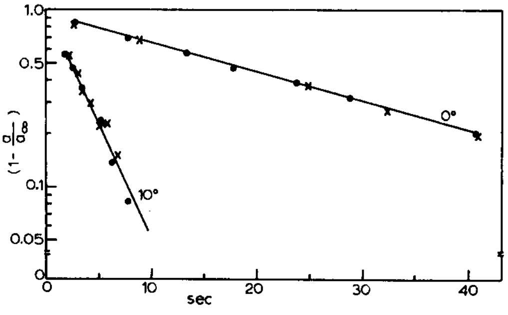

Fig. 1. The rates of steady state exchange of chloride between human red cells and bicarbonate-buffered electrolyte medium at 0 and 10° (pH 7.40). The electrolyte medium had the following composition (mM): Na^+, 142; K^+, 3.7; Ca^{2+}, 1.5; Mg^{2+}, 1; Cl^-, 126.5; HCO_3^-, 22; phosphate, 1.1; and glucose, 5. The cells were loaded with $^{36}Cl^-$ during incubation in the medium at pH 7.40 and at the appropriate temperature. The rate of chloride exchange was followed by an erythrocyte filtration technique[3]. The appearance of $^{36}Cl^-$ in the extracellular phase was determined after injecting approx. 200 mg of packed, labelled erythrocytes into 40 ml of vigorously stirred electrolyte medium, kept at constant temperature and pH. The rate constant of chloride exchange was directly determined from the slope of $[1-(a/a_\infty)]$ *vs.* time, a being the extracellular activity at the time of sampling, a_∞ the extracellular activity at isotopic equilibrium. The rate of chloride exchange was accelerated by a factor of seven by the increase of temperature, the rate constants being 0.039 sec^{-1} (0°) and 0.300 sec^{-1} (10°). The results at 10° were obtained in 14 experiments on cells from the donors: ×, M.D.; and ●, J.W. It was determined from the liberation of haemoglobin to the medium that less than 0.5 % of the cells haemolyzed during experiments.

1 2) kcal/mole, corresponding to the Q_{10} of 7, was calculated by linear regression analysis.

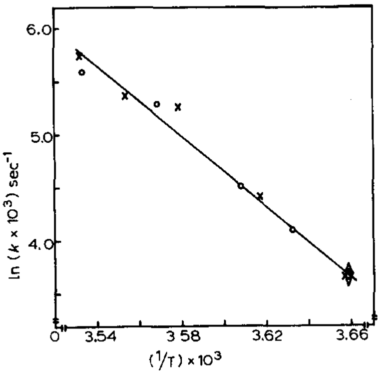

Fig. 2. Arrhenius diagram of the natural logarithm of the rate constant of chloride exchange (k) *vs.* the reciprocal absolute temperature ($1/T$) in the interval between 0 and 10°. Each point represents an experiment of the type shown in Fig. 1. Red cells from 5 donors were used in this study. By linear regression analysis it was found that the rate of chloride exchange between 0 and 10° follows the relation: $\ln (k \cdot 10^3) = -16.7\ (10^3/T) + 64.9\ (r = -0.992)$.

The small amount of information existing about the rate of chloride fluxes in human red cells at low temperatures concerns the exchange of chloride for bicarbonate[2]. The discrepancy between the slow chloride exchange found by us and the one reported previously[2] could be explained if the exchange of Cl^- for HCO_3^- takes place at a greater rate than the self-exchange of chloride isotopes. This is not the case, for we have determined the rate of chloride exchange between cells, equilibrated at 0° and pH 7.40 in a medium containing 126 mequiv chloride and 22 mequiv bicarbonate per liter, and a medium containing 6 mequiv chloride and 142 mequiv bicarbonate per liter (pH 7.40, 0°). The rate constant of chloride exchange was 0.025 sec⁻¹ (S.D., 0.0002), a value significantly lower than the rate constant of chloride exchange between erythrocytes and the plasma-like medium (0.039 sec⁻¹). The impression that bicarbonate retards chloride exchange was strengthened by the observation that the $^{36}Cl^-$ exchange was 1.6 times faster at 0° in bicarbonate-free phosphate-buffered medium[1], than in the presence of 22 mequiv bicarbonate per liter.

TABLE I

SODIUM FLUXES OF HUMAN RED CELLS BETWEEN 0 AND 38°

Influx and net flux of sodium in human red cells incubated at pH 7.40 at a relative cell volume of 0.25–0.35 in a bicarbonate-buffered Ringer solution containing 142 mequiv sodium per liter (*cf.* legend of Fig. 1). The cell suspension contained ouabain: $3 \cdot 10^{-4}$ mole/l. The sodium influx was determined from the net flux and the accumulation of $^{22}Na^+$ in the cells[7].

Temperature (°)	*Duration of the expt.* (*h*)	*Sodium influx*	*Sodium net flux*
		(*mequiv/kg cell*	*solids per h*)
0	20	0.72	0.59
5	19	0.42	0.37
10	23	0.56	0.41
18	22	0.97	0.59
28	25	2.26	1.35
38	3	7.04	3.96

Thus, a high Q_{10} for the chloride exchange at low temperatures is not a unique feature of the chloride exchange occurring between red cells and salicylate containing media[4]. Therefore it has been examined whether passive cation movements also in a normal plasma-like medium have the paradoxical temperature dependence that has been observed in media containing thiocyanate or salicylate[5]. Very small variations of sodium influx are found at temperatures between 0 and 18° (Table I). Sodium influx and net flux into ouabain-treated red cells has a minimum value of 0.42 mequiv/kg cell solids per h at 5°. Only above 18° does sodium influx increase with a Q_{10} of approx. 2–3, which has been found previously between 24 and 37°, the only temperature range studied so far[6].

TABLE II

SELECTIVE PERMEABILITY AT 0, 5 AND 10°

A comparison of chloride and sodium permeabilities in human red cells between 0 and 10°. The permeabilities were calculated from the unidirectional ion movements: $P_j = M_j/(c_j \cdot f_\psi)$, where P_j is the permeability coefficient (cm/sec), M_j is the flux of the ion considered (mole/cm^2·sec), c_j is the concentration of ion-species j (mole/ml), and f_ψ is the factor correcting for the effect of the electrical field on the sodium influx and on chloride efflux[8]. The membrane potential ($V_m = \psi_i - \psi_o$) was calculated[9] from the equilibrium distribution of radioactive chloride between the water phases of cells and medium (rCl). The calculation of fluxes in mole/cm^2 per sec was carried out assuming a cell area of 162 μ^2, and a normal number of $3 \cdot 10^{13}$ cells per kg cell solids.

Temperature (°)	*rCl*	*Calculated V_m (mV)*	P_{Cl} *(cm/sec)*	P_{Na} *(cm/sec)*	*Selectivity* (P_{Cl}/P_{Na})
0	0.80	−5.3	$1.4 \cdot 10^{-6}$	$2.6 \cdot 10^{-11}$	$5.4 \cdot 10^{4}$
5	0.785	−5.8	$3.8 \cdot 10^{-6}$	$1.5 \cdot 10^{-11}$	$2.6 \cdot 10^{5}$
10	0.769	−6.4	$1.1 \cdot 10^{-5}$	$2.0 \cdot 10^{-11}$	$5.7 \cdot 10^{5}$

The available data are that the chloride permeability of red cells is 10^6 times larger than the alkali cation permeability. However, the present findings show that the selective permeability of human red cells to small cations and anions varies with temperature. Calculating the membrane potential from the equilibrium distribution of chloride, we have determined the permeability coefficients of chloride and sodium at 0, 5 and 10° (Table II). The calculations show that the selectivity of the red cell membrane decreases with temperature in a normal bicarbonate-buffered Ringer solution, the ratio P_{Cl}/P_{Na} being "only" 50000 at 0°. The decrease of selectivity is due almost exclusively to the reduction of chloride permeability with decreasing temperature.

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Received October 2nd, 1970