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Anion permeability of mammalian red blood cells: Possible relation to membrane phospholipid patterns

Red blood cells from various mammalian species differ considerably in their membrane permeabilities to hydrophilic nonelectrolytes such as glycerol or erythritol[1-4]. These differences have been discussed in terms of membrane pores[3,4] and in the light of membrane phospholipid patterns[1,4]. In contrast, ion permeabilities of mammalian erythrocytes have not been compared systematically. Different rates of net anion exchange (Cl^-/SO_4^{2-}) were observed by MOND[3] in red cells of man, pig, horse and ruminants. From a quantitative evaluation of such exchange studies, PASSOW[5] concluded that the mechanism of passive anion transfer in pig erythrocytes might not be identical with that in red cells of horse and ox.

The kinetics of net anion exchange in the red cell, however, are influenced by concomitant changes of the membrane potential[6]. Thus, data from experiments of this type are not necessarily conclusive with respect to anion permeability. In order to provide comparable data for different species, we have studied anion transfer at Donnan equilibrium using orthophosphate as a model anion. Red cells were isolated from fresh heparinized blood and phosphate transfer was measured by means of ^{32}P as described elsewhere[7].

As is shown in Table I, the rates of phosphate uptake differ remarkably in red cells from various mammalian species. These data, however, are not directly comparable, since surface area and volume of the cells and their total number per g differ from species to species. The rates of uptake were therefore converted into fluxes by relating them either to known values of surface area or to values calculated by Emmons' formula (*cf.* ref. 8).

Phosphate fluxes principally follow the same order as the rates of uptake, being lowest in the red cells of ruminants and highest in those of rodents and dog. This sequence of species is not correlated to that observed with respect to glycerol or erythritol permeabilities[1-4]. In contrast, data on net anion exchange[3,5] qualitatively agree with our results.

To clarify whether the processes involved in the passive transfer of anions are the same in all species, the influence on phosphate uptake of temperature, pH and extracellular phosphate level was investigated. Temperature coefficients proved to be high in all species (Table II, column 1). Lowering of pH from 7.35 to 6.8 generally increased phosphate uptake by a factor of about 2 (column 2). At high values of pH (7.6–8.0) phosphate uptake was diminished in all species to the same extent previously observed in human red cells[9]. Elevation of extracellular phosphate concentrations produced an exponential increase of phosphate fluxes in all cases (*cf.* refs. 7, 9). Between 10 and 90 mM uptake rises about 20-fold (column 3). Furthermore, phosphate transfer in all species can be inhibited by amphiphilic compounds such as salicylate or tetracaine (column 4), already shown to be effective in human red cells[9]. These similarities strongly suggest that in all species the transfer of phosphate and probably of anions in general is a diffusional process modified by the same type of interactions between membrane components and penetrating anions.

Transmembrane fluxes of anions under equilibrium conditions depend on mem-

TABLE I

RATES OF PHOSPHATE TRANSFER IN RED BLOOD CELLS FROM VARIOUS MAMMALIAN SPECIES

Incubation medium: 130 mM NaCl, 10 mM sodium phosphate, 20 mM glucose, pH 7.35, cell volume 10%.

Species	*Phosphate uptake (nmoles/g erythrocytes per min)*	*Phosphate influx (pmoles/cm² per min)*	*n*
Rat	213 ± 24	12.9 ± 1.5	5
Guinea pig	164 ± 23	12.4 ± 1.7	6
Dog	143 ± 28	10.6 ± 2.1	8
Man	113 ± 12	7.7 ± 0.8	13
Pig	81 ± 3	6.3 ± 0.6	7
Horse	50 ± 4	2.6 ± 0.2	4
Cat	41 ± 8	4.1 ± 0.7	4
Ox	24 ± 6	1.7 ± 0.4	7
Sheep	24 ± 6	1.3 ± 0.3	6

brane resistance as well as on intramembrane anion concentrations[6]. Recent studies have provided evidence that in the red cell intramembrane concentrations of anions may be determined by the density of positive dissociable fixed charges within the membrane[6]. Varying densities of these fixed charges could be one reason for different rates of phosphate transfer in mammalian red cells. In order to test this hypothesis, fixed charge concentrations were estimated using a procedure devised by PASSOW[6]. According to our results the densities of fixed charges controlling intramembrane anion concentrations may be assumed to be similar in all red cells studied (2.5–3 moles/l membrane water, pK approx. 9).

Thus, the species differences in phosphate transfer can be ascribed to differences in membrane resistance, the reasons for which remain to be elucidated. Theoretically, different radii of membrane pores might be responsible. The few data avialable (ox 4.1 Å, man 3.5–4.2 Å, dog 6.0 Å (*cf.* ref. 10)), however, do not favor this assumption. Differences in membrane composition are more likely to be involved, since phosphate

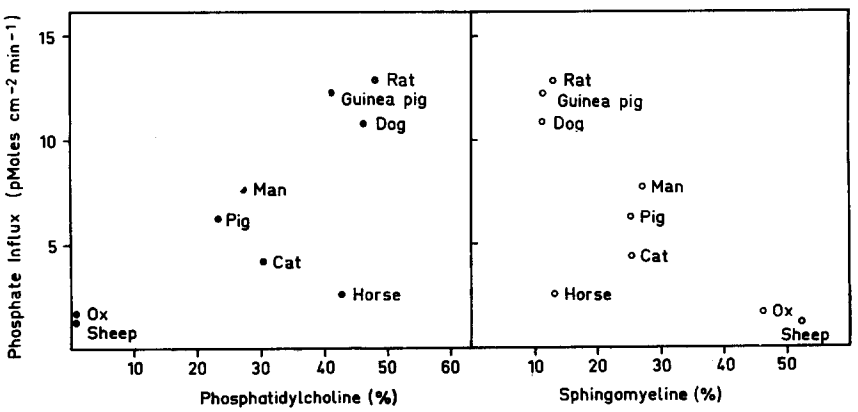

Fig. 1. Relationship between phosphate permeability and membrane phospholipids in mammalian red blood cells. Phospholipid values, taken from NELSON[11], are expressed as percentage of the sum of phospholipids.

TABLE II

CHARACTERISTICS OF PHOSPHATE TRANSFER IN MAMMALIAN RED BLOOD CELLS

Species	$Q_{10} = \frac{Flux\ 37°}{Flux\ 27°}$ (pH 7.35, $PO_4^{3-}{}_e$ = 10 mM)	$\frac{Uptake\ (pH\ 6.8)}{Uptake\ (pH\ 7.35)}$ ($PO_4^{3-}{}_e$ = 10 mM, 37°)	$\frac{Uptake\ (PO_4^{3-}{}_e = 90\ mM)}{Uptake\ (PO_4^{3-}{}_e = 10\ mM)}$ (pH 7.35, 37°)	*Inhibition of PO_4^{3-}-efflux by*	
				Salicylate 5 mM (%)	*Tetracaine 1 mM* (%)
Rat	4.0	2.40	18.5	75	55
Guinea pig	4.75	2.00	20.3	70	54
Dog	4.0	2.18	19.8	68	72
Man	4.9	2.04	17.5	75	64
Pig	4.65	2.06	21.0	60	48
Horse	—*	2.29	19.0	—*	—*
Cat	—*	2.18	20.1	—*	—*
Ox	5.0	2.21	18.8	71	80
Sheep	5.3	2.24	25.0	—*	40

* Not determined.

fluxes could be correlated to the phospholipid patterns of the membranes as determined by NELSON[11]. In general, fluxes increase with the content of the membranes in phosphatidylcholines and decrease with that in sphingomyelines (Fig. 1). As yet, the only exception are horse red cells, in which other factors seem to control phosphate permeability.

Phosphatidylcholines principally are rich in unsaturated medium-chain fatty acids, whereas sphingomyelines contain a high percentage of saturated long-chain fatty acids. Studies on model membranes have provided evidence that chain length and extent of saturation of the fatty acids of phospholipids may strongly influence membrane permeability. As was shown by DE GIER *et al.*[12] nonelectrolyte fluxes into liposomes prepared from synthetic phospholipids increase when saturated long-chain fatty acids are replaced by unsaturated medium-chain ones. On the basis of these findings our results support the working hypothesis that the anion permeability of the red blood cell is causally related to the phospholipid pattern of its membrane.

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