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Comparative Aspects of Phosphate Transfer across Mammalian Erythrocyte Membranes

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Summary. Magnitude and characteristics of phosphate transfer through the erythrocyte membranes of ten mammalian species were measured using tracer exchange techniques. Remarkable quantitative species differences could be demonstrated, permeabilities (at an extracellular phosphate concentration of 10 mM) increasing from 0.2×10^{-8} cm/sec (sheep) to 2.2×10^{-8} cm/sec (rabbit) in the sequence sheep < ox < cat < horse < pig < man < dog < guinea pig < rat < rabbit. In contrast, the characteristics of the phosphate transfer system, such as temperature dependency, dependency on anion composition and pH of the media and sensitivity to amphiphilic inhibitors proved to be very similar in all species, suggestive of a uniform mechanism of transfer. The quantitative differences in permeability which roughly parallel those reported for a number of nonelectrolytes could be correlated with the phosphatidylcholine and sphingomyeline contents of the membranes. The possible molecular basis of a causal relationship between phosphate permeability and phospholipid patterns is discussed.

The characteristics of the slow, passive transfer of divalent anions across the human erythrocyte membrane have been elucidated in a number of comprehensive studies during the last years [10, 30, 46]. Recently, the detailed analysis of rapid movements of monovalent anions such as the halides and SCN^- has also become feasible due to improved techniques of rapid sampling [4, 11, 46]. Thus, detailed information on the anion permeability of the human erythrocyte is already available.

In contrast, only very little is known on anion transfer in erythrocytes of other mammalian species. In early studies [27], the rates of net exchange of extracellular sulfate with intracellular chloride were found to increase in the order ox < sheep < pig < man. From different time courses of sulfate/chloride exchange in pig and ox erythrocytes it was later concluded [16] that different mechanisms might be involved in the net transfer of divalent anions across the erythrocyte membranes of these two species. Such differences would not be too surprising in the light of some other well-established

species dissimilarities concerning both, active and passive transfer processes in erythrocytes [18, 22, 42, 45].

To clarify whether species-specific dissimilarities are also present in case of anion transfer, and to provide further data on the mechanism of this transfer process, a comparative study of phosphate transfer in nine animal species was undertaken. The results, briefly reported in preliminary communications [13, 14], favor the assumption of a common mechanism of phosphate transfer in the erythrocytes of all the species studied in spite of remarkable quantitative differences, the possible reasons of which will be discussed.

Materials and Methods

Blood from laboratory animals was obtained by venipuncture (dog), heart puncture (cat) or exsanguination from V. jugularis and A. carotis (rabbit, guinea pig and rat). Blood from three to five animals was usually pooled in the cases of the smaller animals. Ox, sheep and pig blood was obtained from the local slaughterhouse. Horse blood was obtained from conscious animals by venipuncture.

After addition of heparin (1 mg/ml) and filtration through cotton wool, the specimens (30 to 50 ml) were immediately centrifuged (4 min at 4,500 $\times g$). Plasma and buffy coat were removed by aspiration and the red cells washed twice with a threefold excess of Locke solution [9]. An aliquot of the deproteinized plasma served for the determination of inorganic phosphate concentrations. Phosphate transfer was studied by measuring either the influx or the efflux of ^{32}P-orthophosphate at anion equilibrium.

Phosphate Uptake Experiments

The erythrocytes were suspended at a hematocrit of 10% in media prepared from 125 mM sodium phosphate (Na_2HPO_4/NaH_2PO_4) and isotonic solutions of sodium salts of monovalent (154 mM) or divalent (100 mM) anions, mixed in adequate proportions to give the concentration required in the experiment. Glucose (20 mM) was added to all media. The pH of the suspensions was adjusted to the desired value by addition of 0.3 N NaOH or HCl.

To allow all anions present, including phosphate, to equilibrate between cells and medium, the suspensions were then incubated under gentle shaking in a water bath at 37 °C for time periods determined empirically. At the end of this preincubation period ^{32}P-orthophosphate (5 μC/ml of suspension) was added. After 2 to 7 min of exposure the uptake of isotope was interrupted by rapid cooling of the suspension to 0 °C.

The subsequent determination of the total radioactivity of the cells (cpm/g) and the specific activity of the extracellular medium (cpm/μmole P) as well as the calculation of the rate of phosphate uptake (μmoles P/g cells/min) were carried out as described previously [9].

Phosphate Release Experiments

The washed erythrocytes were incubated (90 min, 37 °C) at a hematocrit of 12% in the following medium ("A"): NaCl 138 mM, sodium phosphate 10 mM, glucose 10 mM, pH 7.35. This initial incubation at a low hematocrit served the purpose to equilibrate phosphate ions between cells and medium without major changes of the extracellular

phosphate concentration. Subsequently, the suspension was centrifuged (6 min at 4,500 × *g*) and the hematocrit increased to 50% by removing an appropriate amount of extracellular medium. The concentrated suspension was then exposed to ^{32}P-orthophosphate (5 μC/ml) for 45 min. The ^{32}P-loaded cells were separated from the medium and washed three times with an excess of isotope-free medium "A" at 0 °C.

For the measurement of ^{32}P release, the cells were mixed with fresh medium "A" (hematocrit 20%). This suspension was immediately subdivided into smaller portions, which were either incubated at different temperatures or in the presence of inhibitors of phosphate transfer, added in a very small volume of water. In the cases involving the measurements of phosphate release at different temperatures, the pH of medium "A" was adjusted at the desired temperature before addition of the isotope-loaded cells. No further adjustments of pH were carried out during the release phase.

Aliquots of the suspensions were sampled at 0, 5, 10 and 20 min, chilled to 0 °C and centrifuged rapidly. The radioactivity of the supernatant was measured in a beta-sensitive scintillation well counter. The increase of radioactivity in the medium (cpm/ml/min) served as a measure of the release of phosphate from the cells.

Results

The Absolute Rates of Phosphate Transfer

As a basis for the comparative studies, the absolute rates of phosphate uptake into the erythrocytes of all species investigated were determined under standard conditions at anion equilibrium and an extracellular phosphate concentration of 10 mM. This phosphate level was chosen, since at the physiological plasma phosphate levels, ranging from 1 to 2.5 mM, the rates of transfer may be so low that adsorption of phosphate to the membrane, which can not be distinguished from transfer by our experimental technique, contributes significantly to the total "uptake" of phosphate.

According to the results presented in the left column of Table 1, the rates of phosphate uptake into erythrocytes of man and nine other mammalian species differ considerably, values ranging from about 250 nmoles/g/min in the rabbit to about 25 nmoles in ox and sheep. These transfer rates, calculated per cell weight, cannot be compared directly, since surface area and volume of the single erythrocyte vary considerably from species to species. To overcome this difficulty, rates of phosphate transfer (nmoles/g of cells/min) were related to the cell surface area (cm^2/ml of cells), using the data given in Table 2 and a mean value of cell density of 1.1 g/ml. To put these calculations on a common basis for the different species, all values of surface area were computed by Emmons' formula [17] from data for cell diameter and mean cellular volume in the literature. Since Emmons' formula is based on simplifying assumptions concerning the shape of the red cell [17, 32], the values for surface area thus obtained are subject to an error between 10 and 20%. Nevertheless, even approximate fluxes calculated from these values (Table 1, right column) provide a useful basis for the

Table 1. Uptake and influx of inorganic phosphate ions into erythrocytes from different mammalian species[a]

Species	Uptake (nmoles · g^{-1} · min^{-1})	Influx (pmoles · cm^{-2} · min^{-1})	n
Rabbit	252±72	13.3±3.8	11
Rat	213±24	11.7±1.3	5
Guinea pig	164±23	10.8±1.5	6
Dog	143±28	9.0±1.8	8
Man	113±12	7.5±0.8	13
Pig	81±3	5.4±0.8	7
Horse	50±4	3.2±0.3	4
Cat	41±8	2.8±0.5	4
Ox	24±6	1.6±0.4	7
Sheep	24±6	1.2±0.3	6

[a] Extracellular phosphate level 10 mM; pH 7.35; 37 °C. Mean values ± SD.

Table 2. Morphological parameters of erythrocytes in different mammalian species

Species	Diameter (μ)	Mean cellular volume (μ^3)	Surface area[h] (μ^2)
Rabbit	7.5[a]	57[b]	118
Rat	7.5[a]	61[g]	121
Guinea pig	7.5[c]	77[d]	129
Dog	7.1[a]	67[b]	117
Man	7.9[a]	87[a]	142
Pig	6.0[b]	58[b]	95
Cat	5.8[b]	57[e]	92
Horse	5.5[g]	48[f]	83
Ox	6.0[a]	58[e]	95
Sheep	5.2[a]	30[b]	66

[a] (32); [b] (45); [c] (2); [d] (1); [e] (35); [f] (16); [g] (15); [h] Calculated according to ref. [17].

comparison of rates of phosphate transfer in erythrocytes from different species. Such values may also be compared with the fluxes of phosphate and other solutes across various types of membranes. Moreover, phosphate permeabilities may be computed from the fluxes. These values range from 0.2 to 2.2×10^{-8} cm/sec if the influence of the transmembrane potential is not taken into account in the calculation.

From the data in Table 1 it becomes obvious that phosphate permeability is highest in the erythrocytes of the rodents and lowest in those of the ruminants. The other species range between these extremes. The species sequence obtained in our studies qualitatively corresponds to that reported by Mond and Gertz [27] for sulfate net exchange in several species and also

agrees partly with data of Prankerd and Altman [33]. The relationship to other permeabilities and membrane properties will be considered below.

The Characteristics of Phosphate Transfer

Temperature Dependency. To clarify whether the species differences observed are only quantitative in nature or result from principal differences of the transfer mechanisms involved, the influence of various environmental parameters on the rates of phosphate transfer was analyzed. Temperature coefficients, determined by measuring the release of ^{32}P-orthophosphate at 37 °C and 27 °C, proved to be high (4 to 5) in all the species analyzed (Table 3). The absolute Q_{10} values for the phosphate permeability in the different species may differ to some extent from the data given in Table 3 due to small differences at the two temperatures of intracellular pH, and phosphate distribution between cells and medium. Both parameters will affect phosphate permeability in addition to temperature per se. Irrespective of such possible deviations, it may be concluded from these results that in all the species the activation energy of phosphate transfer amounts to 25 to 30 kcal/mole. Although activation energies do not allow conclusions concerning the nature of a transfer process, their similarity favors the idea of a common mechanism of phosphate transfer in the species investigated.

Concentration Dependency. Phosphate transfer into human erythrocytes increases overproportionally when the extracellular phosphate level is increased at the expense of another anion present, e.g. chloride [9, 10]. This peculiarity could now also be demonstrated for the erythrocytes of other mammalian species. Phosphate influx becomes enhanced 20- to 25-fold, when the extracellular phosphate concentration is elevated from 10 to 90 mM (Fig. 1). As an exception, phosphate transfer in rabbit erythrocytes increases only slightly overproportional (Fig. 2) with the extracellular phosphate concentration.

Table 3. Temperature coefficients of phosphate efflux from mammalian erythrocytes[a]

Species	Q_{10}
Rat	4.0
Guinea pig	4.8
Dog	4.0
Man	4.9
Pig	4.7
Ox	5.0
Sheep	5.3

[a] Q_{10} values calculated from the release of ^{32}P (cpm/min) at 37 °C and 27 °C; pH = 7.35.

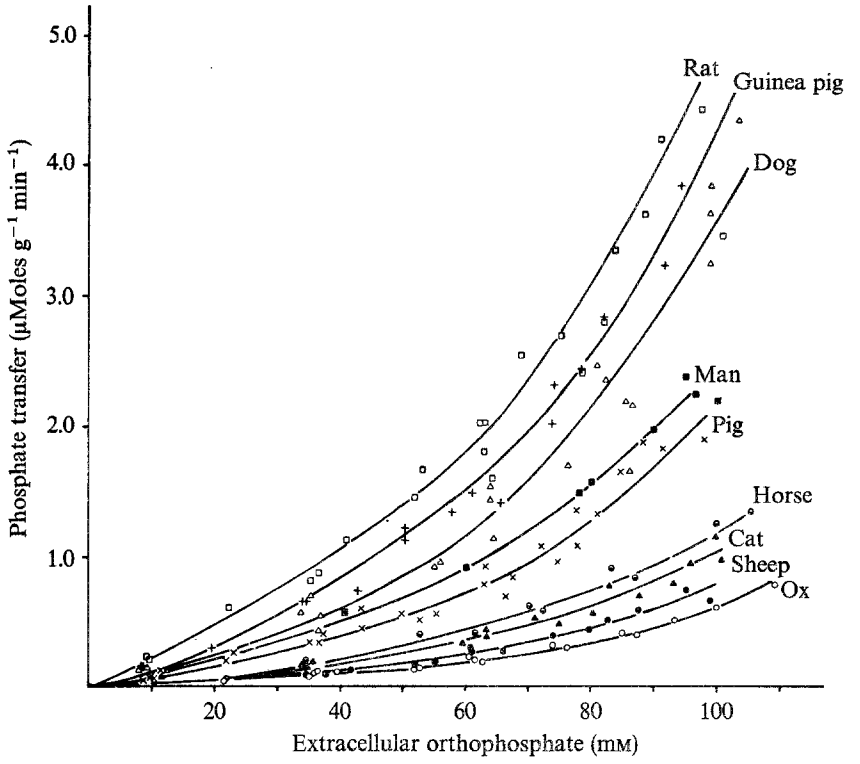

Fig. 1. Nonlinear concentration dependency of the phosphate influx into erythrocytes of different mammalian species. Fluxes determined at anion equilibrium after 90-min preincubation. Incubation media: Mixtures of 154 mM NaCl and 125 mM Na_2HPO_4/NaH_2PO_4 buffer; pH 7.35, 37 °C. Values for human erythrocytes from ref. [10]

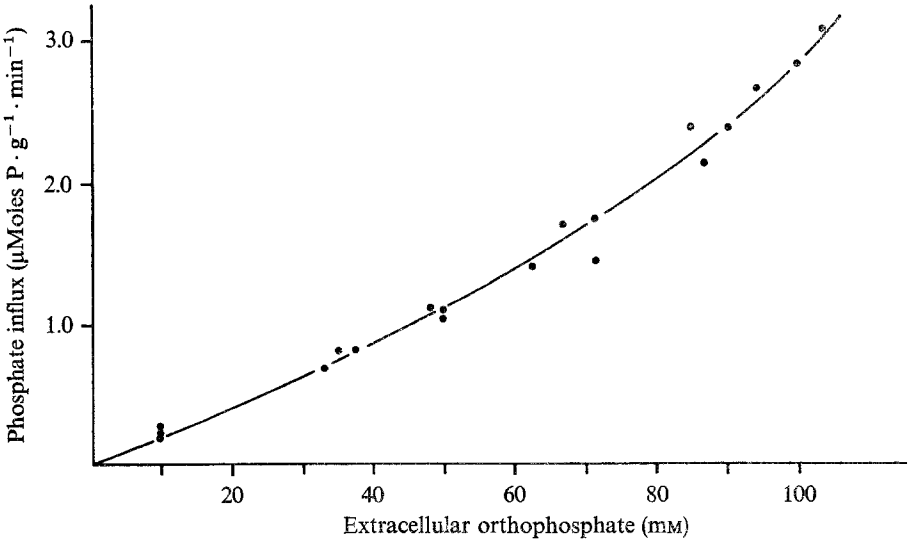

Fig. 2. Concentration dependency of phosphate influx into rabbit erythrocytes. Experimental details as in Fig. 1

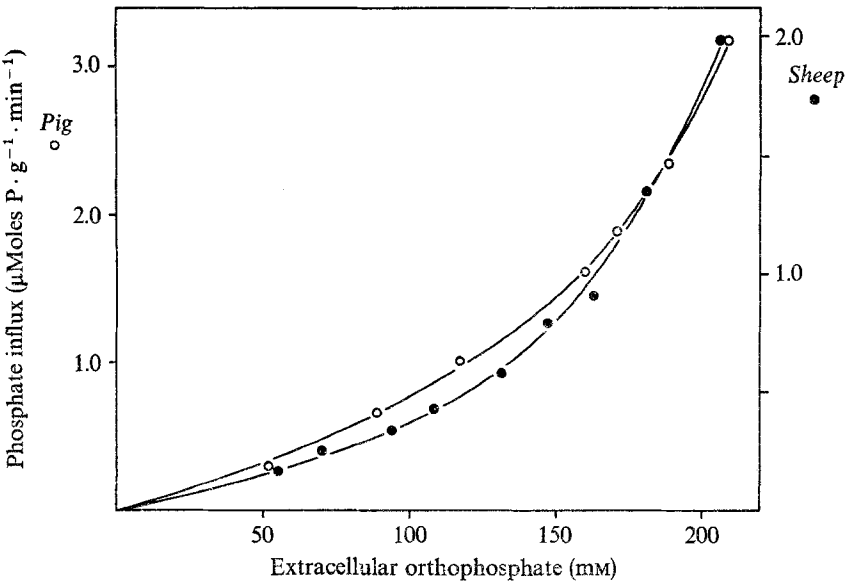

Fig. 3. Overproportional increase of phosphate influx up to maximal extracellular phosphate levels. pH 7.35, 37 °C. For further experimental details *see* Text

It has recently been proposed that the nonlinear concentration dependency of the transfer of phosphate and other divalent anions in human erythrocytes might be due to an increase of membrane permeability induced by cooperative interaction of these anions with membrane subunits [10, 26, 30, 31]. In the light of this hypothesis one might expect that a plot of phosphate influx versus extracellular phosphate concentration would level off at very high phosphate concentrations, since the phosphate "receptors" responsible for the changes of phosphate permeability should finally become saturated with phosphate.

To test this assumption, pig and sheep erythrocytes were incubated in solutions containing up to 210 mM sodium phosphate, the maximal concentration tolerated by the cells without considerable hemolysis. Up to a phosphate concentration of 125 mM, isoosmotic (310 mosm) incubation media were used and the phosphate concentrations raised at the expense of chloride. Above 125 mM, phosphate solutions of increasing osmolarity served as incubation media. After a preequilibration of the cells with their media, ^{32}P uptake was measured and transfer rates calculated taking into account the shrinking of the cells in the hypertonic media. As is evident from Fig. 3, phosphate transfer increased overproportionally up to the highest phosphate levels studied. Thus, there is no indication of any saturable component in phosphate transfer under these conditions.

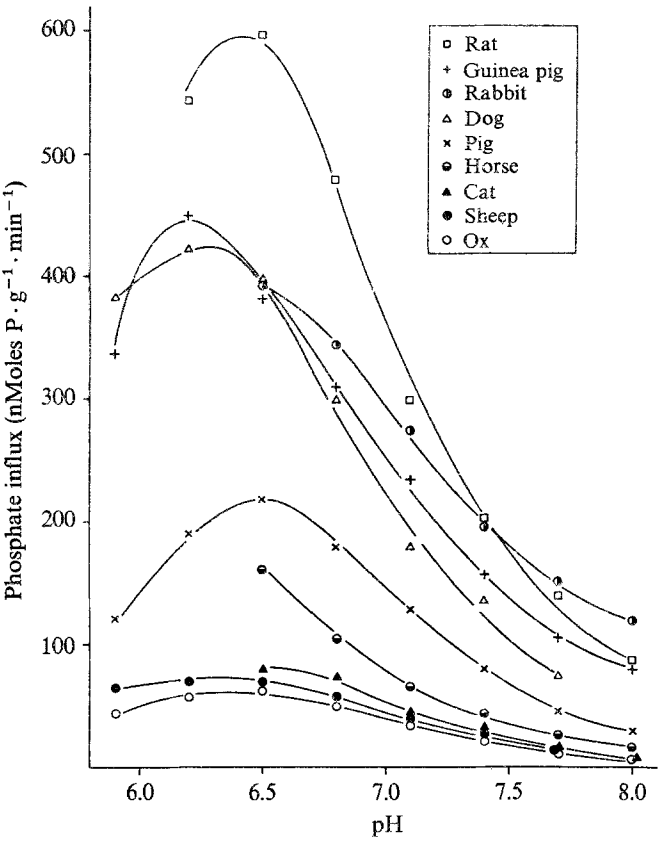

Fig. 4. pH dependency of phosphate influx into erythrocytes from various mammalian species. Extracellular phosphate concentration: 10 mM, 37 °C. Values for human erythrocytes from ref. [10]

pH Dependency. Lowering of the extracellular pH has been shown previously to accelerate remarkably the transfer of phosphate and other divalent anions across the human erythrocyte membrane [10, 30]. A maximum of transfer was observed at about pH 6.4 [10, 36]. From the curves in Fig. 4 it becomes obvious that in all other species the pH dependency of phosphate transfer follows the same pattern. Even the positions of the maxima coincide for those species for which they could be determined without hemolysis. Moreover, quantitative agreement of the pH effects could be demonstrated by converting the transfer rates for each species into arbitrary units relative to the rate at pH 7.40 (Fig. 5).

Influence of Anions. The idea of a common mechanism of phosphate transfer could be further substantiated by studying the influence of anions on phosphate transfer. According to our results, replacement of extra-

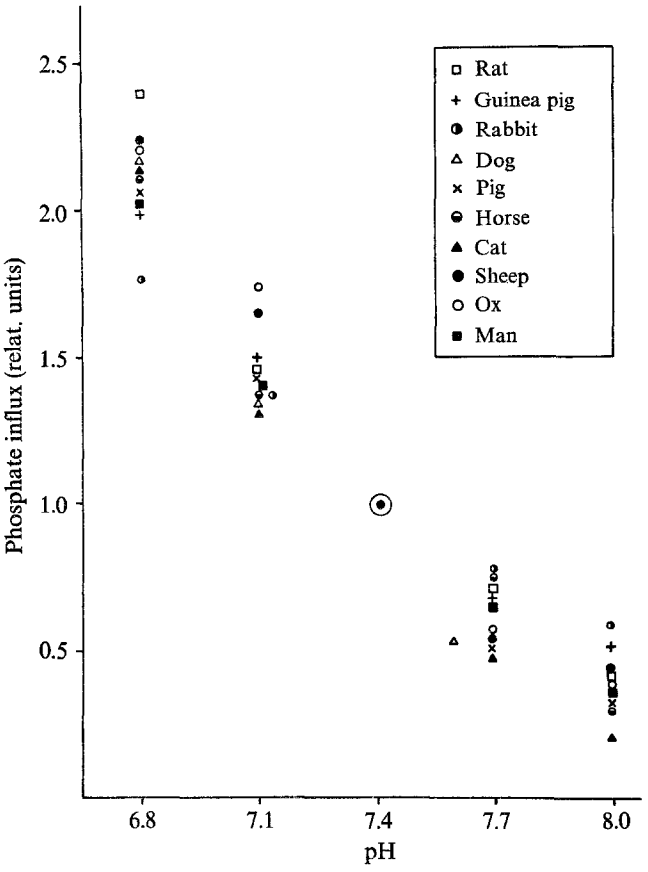

Fig. 5. Quantitative agreement of pH dependencies of phosphate transfer in different mammalian species. Data from Fig. 4 in arbitrary units, relative to the values obtained at pH 7.4

cellular chloride by equiosmolar concentrations of other mono- or divalent anions at a constant extracellular phosphate concentration influences phosphate transfer in all species. With a few exceptions, fluxes increase in the order $SCN < NO_3 < J < Cl <$ acetate $<$ sulfate $<$ lactate $<$ malonate (Fig. 6), previously also demonstrated for human erythrocytes [10].

The Influence of Inhibitors. In addition to its dependency on environmental parameters, the phosphate permeability of the human red cell membrane is characterized by a remarkable sensitivity to numerous amphiphilic compounds which inhibit phosphate transfer at concentrations between 0.005 and 5 mM [10, 31, 38]. As could be demonstrated in the present investigation these compounds also reduce phosphate transfer in

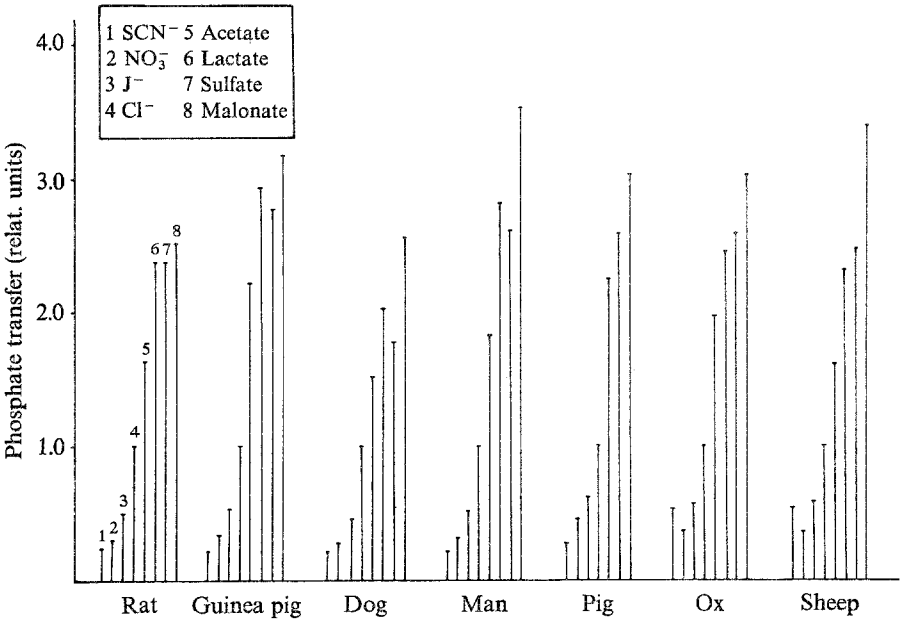

Fig. 6. Influence of the anion composition of the incubation media on phosphate transfer in mammalian erythrocytes. Data in arbitrary units, relative to the transfer rates in chloride media. Incubation media: Isotonic mixtures of Na_2HPO_4/NaH_2PO_4 (10 mM) and the sodium salts of the different anions. pH 7.35, 37 °C. Cells pre-equilibrated with the various anions

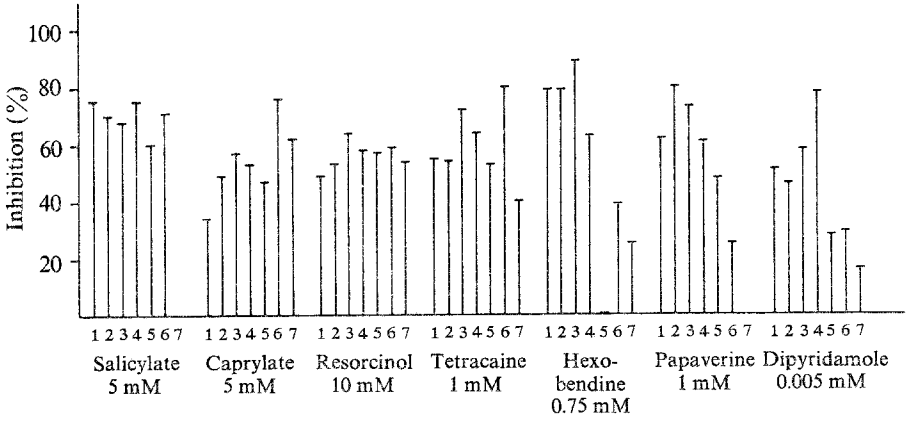

Fig. 7. Influence of amphiphilic inhibitors on phosphate efflux from erythrocytes of different species. *1* Rat; *2* Guinea pig; *3* Dog; *4* Man; *5* Pig; *6* Ox; *7* Sheep

all other species (Fig. 7). The effectivities of anionic and nonionized inhibitors are essentially species-independent. In contrast, several cationic inhibitors proved to be less effective in pig, ox and sheep erythrocytes than

in others. Hexobendine, e.g., is completely ineffective in pig erythrocytes. These partial discrepancies might be explained by different accessibilities or affinities of the membrane sites interacting with the inhibitors.

Discussion

Relationships between Phosphate Permeability and Other Permeabilities

The uniform transfer characteristics demonstrated in our comparative studies suggest that inorganic phosphate ions penetrate the erythrocyte membranes of all mammalian species studied by identical mechanisms similar to those previously discussed for the transfer of divalent anions in human erythrocytes [10, 19, 30, 31, 46, 48]. In this uniformity, the transfer of phosphate differs from that of other solutes.

Na^+ and K^+, e.g., are transported against considerable electrochemical gradients across the erythrocyte membranes of man, pig, rat, rabbit and HK sheep by a glycoside-sensitive transport system [34, 42], which is only slightly developed or virtually absent in the low potassium erythrocytes of ox [21], LK sheep [40], cat and dog [42]. A further example for species differences in solute transfer is provided by polyols such as ethylene glycol, glycerol and erythritol. These nonelectrolytes penetrate the erythrocyte membranes of some species by "simple" diffusion [7, 22] whereas in others facilitated, presumably carrier-mediated diffusion seems to be involved [22, 24, 47].

In view of such differences, a comparison of the species sequence for phosphate transfer with sequences for other solutes will only provide meaningful information when it is restricted to species having the same transfer mechanism for a solute. With this reservation very few data can be considered.

Cation loss from erythrocytes incubated in isotonic sucrose solutions presumably occurs by the same mechanism in all species and may even involve the same positive fixed charges in the membrane which are supposed to determine phosphate transfer [30]. The species sequence for the rates of this process (man > guinea pig > rat > cat > pig, rabbit, ox [5]), however, greatly differs from that for phosphate transfer.

In contrast, a fairly close relationship is obtained, when phosphate permeabilities are compared with nonelectrolyte permeabilities of erythrocytes in which only nonmediated diffusion accounts for the transfer. As is evident from Fig. 8, the rates of ethylene glycol and glycerol penetration increase in the same order as those of phosphate transfer in erythrocytes of

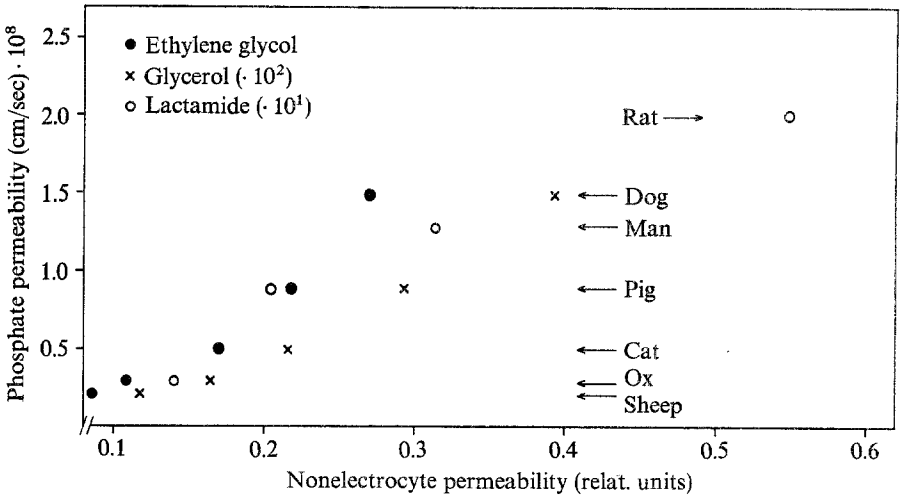

Fig. 8. Realtionship between permeabilities to phosphate and to various nonelectrolytes in erythrocytes from different species. Nonelectrolyte permeabilities (from ref. [20] and ref. [22]) as reciprocals of hemolysis times (relative to hemolysis time in 20 mM NaCl) in presence of the nonelectrolyte

sheep, ox, cat, pig and dog which have no carrier system for the polyols in contrast to the erythrocytes of guinea pig, rabbit and man [22]. A satisfactory parallelism can also be demonstrated between the rates of penetration of lactamide [20] and those of phosphate for the erythrocytes from ox, pig, man and rat. These correlations may suggest that the same barrier limits the transfer of phosphate and of nonelectrolytes.

Possible Reasons for the Species Differences in Phosphate Permeability

"Intramembrane" Concentrations of Phosphate. The rate of the "simple" diffusion of a solute across a membrane in general depends on (1) the partition of the solute between membrane and extramembrane phase, which determines its "intramembrane" concentration gradient, and (2) the mobility of the solute within the membrane. Species differences in the rates of phosphate transfer might be ascribed to either of these two factors, the experimental discrimination of which is impossible for biomembranes as yet. In the case of the erythrocyte membrane, evidence has been presented [10, 26, 30, 46], that the partition coefficients for anions might depend on the concentration of positive, dissociable fixed charges in the membrane. The anion partition was supposed to be governed either by a simple Donnan

distribution [10, 26, 30] or by complicated ion exchange equilibria [46]. The former assumption made the hypothetical fixed-charge density and thus partition coefficients for anions accessible to a quantitative estimation.

By an involved procedure [26, 30], based on the postulate of a unique pH-independent relationship between phosphate fluxes and intramembrane phosphate concentrations, a density of 2.5 to 3 moles/liter H_2O and a dissociation constant of 9.0 were assigned to the hypothetical fixed charges controlling the transfer of sulfate [30] and phosphate [10] across the human erythrocyte membrane. The same values resulted when this procedure was applied to the data obtained in the above studies on phosphate transfer in the different mammalian erythrocytes (Gruber, M. D. Thesis, 1973), indicating identical partition coefficients of phosphate in all species.

A single type of positive fixed charges, however, can only account for the rediminution of phosphate permeability at pH values below 6.3 (*cf.* Fig. 4) if additional assumptions are introduced. Moreover, the recently established pH dependency of the transfer of monovalent anions [11, 19, 48] is opposite to that of divalent anions between pH 7 and pH 8 and therefore irreconcilable with the "classical" fixed charge concept. For this and other reasons a titratable carrier system for inorganic anions has recently been postulated in the erythrocyte membrane [19, 48]. In terms of this model, equal partition coefficients of phosphate would require similar concentrations and phosphate affinities of the carrier in all species. Our data, especially the coinciding pH dependencies are suggestive of such similarities but do not yet provide unequivocal evidence. The question whether differences in the partition of phosphate between membrane and extramembrane phase contribute to the species differences in phosphate transfer will probably have to wait for its final answer until the basic mechanisms of the transfer of phosphate are better understood.

Membrane Composition and Phosphate Transfer. Species differences in phosphate transfer could also be due to different mobilities of phosphate ions traversing the membranes either free or bound to a carrier molecule. Such differences in mobility might result from differences in membrane composition. The comparative data available in this respect justify the attempt of a correlation with membrane permeability.

Membrane proteins of some species exhibit striking similarities [23, 25, 29]. For other species characteristic dissimilarities of protein composition have been reported [50]. However, neither the similarities nor the differences bear any relationship to the rates of phosphate transfer in the different animal species.

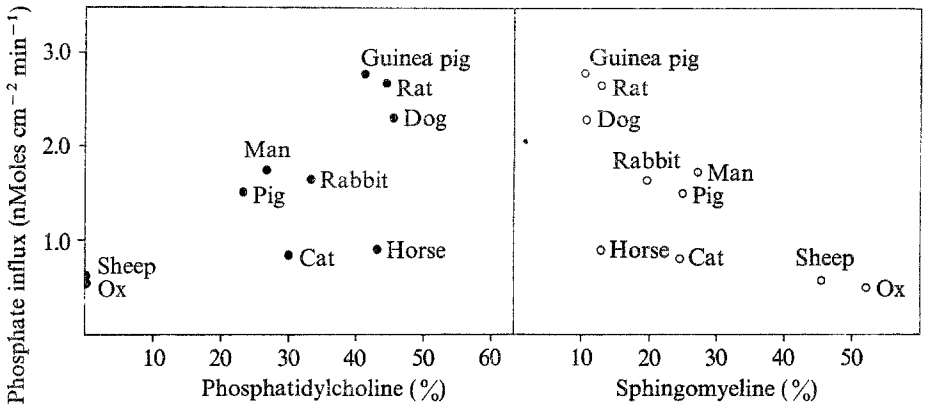

Fig. 9. Relationship between phosphate transfer and membrane phospholipids in erythrocytes from different species. Transfer rates measured at an extracellular phosphate level of 95 mM, pH 7.35, 37 °C. Lipid concentrations (as % of the total phospholipids) from Nelson [29]

As a second group of membrane constituents, lipids have to be considered in connection with permeability. Comprehensive data for the concentrations of these compounds in mammalian erythrocyte membranes have been provided by Nelson [29]. From his results it follows, that neither the total lipid concentrations (per μ^2 of cell surface area), nor the total concentrations of phospholipid or cholesterol, which all differ from species to species, can be correlated with the rates of phosphate transfer. The same is true for the phospholipid/cholesterol ration, which is very similar in all species.

If one takes into consideration, however, the distribution patterns of single phospholipids, interesting relationships can be demonstrated. Erythrocyte membranes from various mammalian species differ widely in their contents in phosphatidylcholines and sphingomyelines [29]. These variations are paralleled by the differences in phosphate transfer in a systematic way. As is evident from Fig. 9, the transfer rates are correlated positively with the phosphatidylchloline content of the membranes and negatively with that in sphingomyelines. As exceptions, cat and horse erythrocytes do not fit into this scheme, having a phospholipid composition characteristic of more permeable cells.

To clarify whether these correlations reflect a causal relationship, structural peculiarities of the two classes of phospholipids have to be compared. Both have the same functional organization of the polar moiety of the molecule in spite of different chemical constitution. The apolar parts of the two amphiphilic molecules, however, differ in their structure. Phosphatidylcholines contain a high amount of fatty acids with intermediate chain

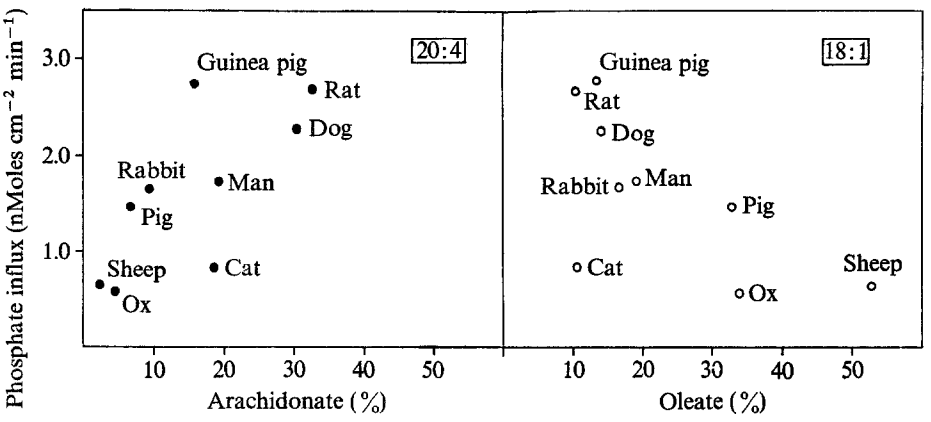

Fig. 10. Relationship between phosphate transfer and contents of two fatty acids in erythrocyte membranes. Transfer rates as in Fig. 9. Fatty acid values (in % of total fatty acids determined) from De Gier (*Personal communication*)

length and two or more double bonds, whereas sphingomyelines are characterized by the occurrence of fatty acids with chain lengths >20 and a very low number of double bonds [37].

If the species differences in phosphate transfer were due to these dissimilarities in fatty acid composition, fluxes should also correlate with the membrane concentrations of characteristic fatty acids. Evaluation of our transfer data under this aspect (Fig. 10) in fact reveals a positive correlation of fluxes with the membrane contents of a highly unsaturated fatty acid (arachidonate) and a negative correlation with a shorter and more saturated one (oleate), although some exceptions indicate the contribution of other parameters to the magnitude of phosphate permeability.

In recent comparative studies a positive correlation between the degree of unsaturation of membrane fatty acids and glycerol permeability could be demonstrated [6]. This obvious correspondence between phosphate and glycerol permeability provides further support for the assumption, that the same barrier limits the transfer of phosphate and of nonelectrolytes. The importance of the alkyl chains of fatty acids, i.e. of a hydrophobic region of the erythrocyte membrane, for the transfer of hydrophilic and probably even charged molecules is not unlikely in view of results obtained with phospholipid bilayers and with the microorganism, Mycoplasma laidlawii B. In these two systems, permeability has been clearly shown to increase with rising number of double bonds and to decrease with increasing chain length of the fatty acid moiety [8, 36].

Direct proof for a dependency of phosphate permeability on membrane fatty acid composition would require the investigation of erythrocytes with

different fatty acid patterns but otherwise unaltered membrane composition. Although the fatty acid patterns of erythrocyte membranes can be altered by feeding animals with diets deficient in unsaturated fatty acids [41, 44], it is not possible by this procedure to replace unsaturated acids by saturated ones. Hence, the permeability data obtained on such cells or artificial membranes prepared from their lipids [3, 28, 43] cannot be evaluated in terms of the influence of fatty acid saturation on solute transfer through the erythrocyte membrane. Other techniques for the modification of membrane fatty acids therefore have to be developed for an ultimate elucidation of the significance of fatty acids for the phosphate permeability of the mammalian erythrocyte membrane.

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