Exchange Diffusion in Human Red Blood Cells (36414)

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The concept of exchange diffusion was devised by Levi and Ussing (1) to account for the observation that tracer sodium efflux from resting frog muscle would have required over 30% of its basal oxygen consumption. Later reports were consistent with the view that a portion of tracer movement across biological membranes was due to a carrier-mediated, nonendergonic process (2, 3). Part of the passive movement of cations across red blood cell membranes also was ascribed to an exchange diffusion process (4, 5). However, in 1966 data were presented which seemed incompatible with the view that an exchange diffusion for sodium existed in human red blood cells (6). Garrahan and Glynn (7) subsequently reported evidence supporting a ouabain-sensitive exchange diffusion process; other reports suggested that a ouabain-insensitive exchange diffusion of sodium also occurred in human red blood cells (8, 9). The present data support the view that exchange diffusion is one of the processes which contributes to transmembrane cation movement in human red blood cells.

Methods. Experiments were performed either on outdated, 3- to 4-week-old blood stored in ACD solution, which was obtained from the Barnes Hosital Blood Bank, or on fresh blood drawn from volunteers into heparinized tubes. The methodology for performing sodium influx studies and for the preparation of red blood cells with varying internal sodium concentrations was the same as that previously described (8). Incubations were carried out in duplicate or triplicate at 37° for 1 hr in a shaking water bath. ^{24}Na was counted in a Packard Auto-Gamma counter (Model 5213); a minimum of 10,000 counts was obtained on all samples. A Cotlove chloridimeter was used to measure chloride concentrations in red blood cells and media. The aliquots of cells used for chloride determination were washed with cold isotonic magnesium sulfate. Sodium and potassium concentrations were measured using an IL flame photometer (Model 143), and hemoglobin concentrations were measured by the method of King (10). The uptake of ^{14}C fructose by red blood cells was also measured. In these experiments, an aliquot of the washed, precipitated cell suspension was counted in a Packard Tri-Carb liquid scintillation counter (Model 3224). Standard statistical procedures were employed in analyzing the data.

Results. In each of six experiments on stored blood, tracer influx of sodium was higher in red blood cells with higher internal sodium content (Fig. 1). The difference in influx between the high and low sodium cells averaged 0.32 ± 0.04 μmole/ml of RBC/hr under control conditions and 0.29 ± 0.03 μmole/ml of RBC/hr in the presence of ouabain. The ^{24}Na influx in the high sodium cells was significantly higher than the ^{24}Na influx in the low sodium cells ($p < .001$). Similar results were obtained when fresh red blood cells were used (Fig. 2). Ouabain *per se* had no significant effect in the magnitude of ^{24}Na influx. The acceleration of tracer influx in a potassium-containing medium in the presence of ouabain is consistent with a passive process. That portion of tracer influx which related to internal sodium concentration was approximately 15% of the total ^{24}Na influx, a value similar to that previously found (8).

Since a change in potential across the red cell membrane could have affected the passive transfer of sodium into the cell, the ratio

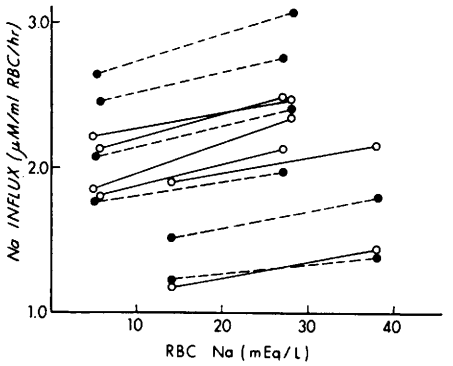

FIG. 1. Effect of internal Na content on ^{24}Na influx in stored blood. The media contained Na, 140 mM; K, 10 mM; P, 20 mM (pH 7.5); inosine, 10 mM; and adenosine, 5 mM. Incubations were for 60 min at 37°. (O—) control conditions; (● - -) ouabain, 0.1 mM.

of red blood cell chloride concentration to medium chloride concentration was determined in several experiments in which the influx measurements were made. These results are shown in Table I. The ratio of internal chloride concentration to medium chloride concentration averaged 0.68 ± 0.03 and 0.70 ± 0.06 in the low and high sodium cells, respectively. Similar ratios were obtained in the presence of ouabain. The transmembrane potential calculated from these ratios approximates 10 mV, a value similar to that previously reported from chloride ratio calculations (11) or from direct measurements (12). Thus, there was no significant difference in transmembrane potential which could explain the observed difference in ^{24}Na influx.

Experiments were also performed to evaluate the effect of temperature on sodium influx (Table II). The Q_{10} (27 to 37°) for this movement averaged 2.27 in low sodium cells and 2.20 in high sodium cells. From these values it can be calculated that the respective activation energies would be 15.2 and 14.6 kcal/mole. Slightly lower values were found when ouabain was present in the medium. Although deductions regarding mechanisms cannot be made from activation energies alone, the values observed are in keeping with those associated with a passive flux (13).

Discussion. Sodium and potassium movements across red blood cell membranes are generally thought to occur by both active and passive mechanisms (14). The former involve the utilization of energy and usually are oriented against an electrochemical gradient. In red blood cells those components of sodium efflux and potassium influx which are inhibited by cardiac glycosides are examples of active transport. Passive transmembrane transport, by contrast, typically occurs down an electrochemical gradient; the latter provides the driving force for this movement. Under appropriate conditions, the influx of tracer sodium into red blood cells appears to be passive in nature. Conceptually, passive ionic movements may occur either by simple diffusion or may involve combination with a carrier. One example of a carrier-mediated passive transport process is exchange diffusion. This involves the exchange of a radioisotope on one side of the membrane for the stable form of the element on the opposite side of the membrane. No net ionic movement occurs as a result of this process, and the only energy requirements are those involving association and dissociation of the molecule from the carrier. Exchange diffusion may be regarded as a special instance of counter-transport, analogous to that which has been shown to obtain for hexose movement across red blood cell membranes (15).

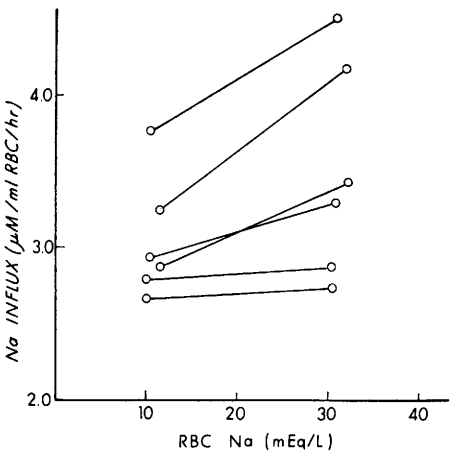

FIG. 2. Effect of internal Na content on ^{24}Na influx in fresh blood. Conditions as in Fig. 1 except that glucose, 10 mM, was employed as substrate.

TABLE I. Chloride Ratios in Red Cells with Different Na Content.
The media contained Na, 140 m*M*; and K, 10 m*M*. Ouabain was used in a final concentration of 0.1 m*M*. Mean values ± SE are given for chloride ratios.

	Control (5)		Ouabain (4)	
	Low Na RBC	High Na RBC	Low Na RBC	High Na RBC
$\frac{[Cl^-]^i}{[Cl^-]^o}$	0.68 ± 0.03	0.70 ± 0.06	0.70 ± 0.07	0.66 ± 0.08
Potential difference (mV)	10.2	9.3	9.3	10.6

In one instance, two isotopes of the same species are exchanged, while in the other, two related but different chemical species are transported by the same carrier.

In the present experiments, the influx of tracer sodium into human red blood cells has been examined with respect to varying internal sodium content. At a given temperature, the unidirectional flux of Na by simple diffusion should be determined by the activity of sodium ions in the medium. It follows that an increase in sodium influx induced by an increase in internal sodium concentration would not appear to be explicable on the basis of diffusional movements alone. The present results, obtained in both stored and fresh human red blood cells, suggest that a carrier-mediated process may be involved in the inward transfer of ^{24}Na across the red blood cell membrane. Since no net change in sodium movement has been demonstrated to occur under these conditions (8), this process seems to fit the definition of exchange diffusion. Several other points support the contention that the accelerated influx of ^{24}Na into high Na cells is, in fact, an exchange diffusion process. First, the transmembrane potential difference, calculated from chloride ratios, was the same in both low Na and high Na red blood cells. Thus, the observed differences in ^{24}Na movement are not attributable to an alteration in the transmembrane electrical potential gradient. Second, the experiments in which the effect of temperature changes on sodium influx was determined demonstrate that activation energies were virtually the same for movement into both high and low sodium red blood cells.

The possibility that a change in membrane permeability in the high and low sodium cells might have been present to account for these differences also was considered. In Table III the values for the uptake of ^{14}C fructose by red blood cells with either high or low sodium concentration are shown. Under both control conditions and in the presence of ouabain, the uptake of ^{14}C fructose per milliliter of red blood cells was not affected by the internal Na content. These results suggest that general differences in membrane permeability in the two groups of cells were not present.

TABLE II. Effect of Temperature on Na Influx.
The media contained Na, 140 m*M*; and K, 10 m*M*. Ouabain was used in a final concentration of 0.1 m*M*.

	Control		Ouabain	
	Low Na RBC	High Na RBC	Low Na RBC	High Na RBC
Q_{10} (27°–37°)	2.27	2.20	1.88	2.00
Activation energy (kcal/mole)	15.17	14.59	11.68	12.82

TABLE III. Uptake of ^{14}C Fructose by Red Blood Cells.

The media contained Na, 140 m*M* and K, 10 m*M* (pH 7.5). The cell suspension was incubated for 1 hr at 37° following the addition of tracer. Results are expressed as counts per minute per milliliter of red blood cells.

	Control		Ouabain	
Expt.	Low Na RBC	High Na RBC	Low Na RBC	High Na RBC
1	3977	4230	4281	4152
2	2767	2578	2533	2862
3	2051	2216	1951	2339
Mean	2932	3008	2922	3118
SE	± 562	± 620	± 700	± 539

The data support the view that a ouabain-insensitive exchange diffusion process for sodium can account for some of the observed tracer Na movement in human red blood cells. These results also support the use of a model in which intracelluar Na is varied to evaluate transport processes. It should be emphasized, however, that the present observations in no way bear the question of the existence of either a "second sodium pump" or an expanded "first pump" (6). In fact, a consideration of red blood cells energetics leaves almost two-thirds of the lacate production unaccounted for (16). The energy derived from this production could well relate to additional active transport mechanisms within the red blood cell.

Summary. Characteristics of a model for the study of diffusional movement in human red blood cells have been examined. Tracer influx of sodium was accelerated in red cells with high internal sodium content. This occurred both in fresh and stored human red blood cells. These results were not due to an alteration in electrical potential across the red blood cell membrane and uptake of a nonpolar tracer was not enhanced by high internal sodium content. The data seem best explained by an exchange diffusion process.

I am grateful to Professor R. Whittam for suggesting the model and to both Professor Whittam and Dr. Neal S. Bricker for reviewing this manuscript. Mr. Foster Harris provided valuable technical assistance. This work was supported by U.S. Public Health Service Grant No. AM 14586-01.

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Received Jan. 17, 1972. P.S.E.B.M., 1972, Vol. 140.