Kinetics and Stoichiometry of Na-Dependent Li Transport in Human Red Blood Cells

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ABSTRACT This paper describes the kinetics and stoichiometry of a tightly coupled Na-Li exchange transport system in human red cells. The system is inhibited by phloretin and furosemide but not by ouabain. Li influx by this system increases and saturates with increasing concentrations of external Li and internal Na and is inhibited competitively by external Na. Comparable functions relate Li efflux and Na efflux to internal and external Li and Na concentrations. Analysis of these relations yields the following values for the ion concentrations required to half-maximally activate the transport system: internal Na and Li 9.0 and 0.5 mM, respectively, external Na and Li 25 and 1.5 mM, respectively. The system performs a 1:1 exchange of Na and Li moving in opposite directions across the red cell membrane. We found no evidence for a simultaneous transport of more than one Na and Li by the system. The maximum transport rate of Na-dependent Li transport varied between 0.1 and 0.37 mmol/(liter of cells x h) in the red cells of the five normal male subjects studied. No significant variations between individual subjects were observed for bicarbonate-stimulated Li transport and for the residual Li fluxes which occur in the absence of bicarbonate and in the presence of ouabain plus phloretin.

INTRODUCTION

In this paper we describe some kinetic characteristics of the Na-Li exchange transport system in human red cells. As has been shown previously, Na-dependent Li transport is insensitive to ouabain and oligomycin, but is completely inhibited by phloretin, furosemide, quinine, and quinidine (Pandey et al., 1978). In the presence of an oppositely directed Na gradient, the system is able to transport Li against its electrochemical potential gradient into or out of the red cells (Haas et al., 1975; Duhm et al., 1976). This Na-dependent Li transport does not require the presence of cellular ATP or K (Pandey et al., 1978). Essentially similar observations have recently been reported by Duhm and Becker (1977 a, b, c) and Duhm et al. (1977). On the basis of these findings we have suggested that one of the mechanisms of Li transport in human red cells is a tightly coupled exchange between Na and Li ions which allows...
transmembrane ion movement only if another ion moves in the opposite direction. We propose that the transport site reacts only with Na and Li, permitting either Na-Na and Li-Li homoexchange or Na-Li heteroexchange. A carrier model of such a system is shown in Fig. 1. The carrier reacts with the metal ion (Na or Li) on one side of the cell membrane and transfers the ion to the other side. Because the unloaded carrier cannot cross the membrane, the system can promote only ion exchange. The rate of ion transport depends on the concentrations of the transported species on both sides of the membrane, the rates of their reactions with the carrier, and the rate of translocation of the ion-carrier complex. Models without mobile carrier molecules (lock-carrier, double-gated channel) yield basically similar kinetic equations (e.g., Gunn, 1977).

\[ M^+ = Li^+ \text{ or } Na^+ \]

**Figure 1.** Carrier model for the tightly coupled exchange of Na and Li ions in the human red cells. (C) Carrier molecule in the membrane; (M) metal ion — either Na\(^+\) or Li\(^+\).

In the present study we have analyzed the kinetics of the Na-Li transport system by altering internal and external Na and Li concentrations. Also, we measured the stoichiometry of the Na-Li exchange, and estimated the maximum capacity of this system in the red cells of five donors.

**Materials and Methods**

The basic materials and methods were the same as described in the preceding paper (Pandey et al., 1978).

**Loading of Red Cells with Li**

To prepare red cells with different Li concentrations and with low internal Na concentrations, the cells were incubated for 3 h in media containing 2-40 mM LiCl substituted for KCl (158-100 mM) plus 10 mM glucose and 16 mM Tris-HCl, pH 7.4 at 37°C. Li concentration in these cells was 0.2-2.5 mmol/liter cells and Na concentration was below 2.5 mmol/liter cells.

**Loading of Red Cells with \(^{22}\)Na**

Red cells were loaded with \(^{22}\)Na either by overnight incubation in a medium containing 140 mM NaCl, 20 mM KCl, and 20 mM Tris-HCl, pH 7.4, at 4°C, or by the rapid loading technique of Sarkadi et al. (1976). In the latter case, red cells (hematocrit 30%) were incubated for 1 min in a medium containing \(^{22}\)Na in 140 mM choline-Cl or 110 mM MgCl\(_2\) plus 20 mM Tris-HCl, pH 7.4, at 37°C, in the presence of 10 \(\mu\)M nigericin. After the loading period, the ionophore was eliminated with four washings in isosmotic choline.
Cl or MgCl₂ media containing 1% albumin at 4°C. Cellular Na or K concentrations did not change during the loading procedure.

**Li Influx Measurements**

Li influx was measured by the red cell separation technique described by Sarkadi et al. (1976). The sample of suspension (0.5 ml) was layered on 12 ml of ice-cold sucrose cushion (0.7 M sucrose dissolved in 0.11 M MgCl₂ buffered to pH 7.4 with 5 mM Tris-HCl). The cushion was then spun for 1 min at 10,000 g. After centrifugation the supernate and the sucrose were removed by suction and the cell sediment was rinsed three times with isosmotic MgCl₂. The washed sediment was dissolved in 1 ml of deionized water and analyzed for ions and hemoglobin. Concentrations in the lysate were related to hemoglobin and the cellular ion concentrations were calculated by using the values for hemoglobin obtained by the nylon tube technique (see Pandey et al., 1978).

**Stoichiometry Measurements**

In this type of experiment red cells were preloaded with ²²Na by overnight incubation. The ²²Na loaded cells were incubated in media containing 1.6-16.0 mM LiCl substituted for KCl (138.4-124 mM) or in all KCl (140 mM) media at 37°C. All media contained 20 mM Tris-HCl, pH 7.4, and 10⁻⁴ M ouabain. The hematocrit was 5%. At three time points (0, 30, and 60 min) 2 ml of suspension was removed and spun for 1 min at 10,000 g. 1 ml of the supernatant was removed, and the sediment was stirred and layered on a sucrose cushion for separation of red cells, as described above. The supernate was analyzed for ²²Na and the cells for Li and Hb. Plots of Li and ²²Na (measured simultaneously) against time were analyzed by linear regression, and the slope of the curve was used for estimating the flux.

In the experiments for studying the kinetics and stoichiometry of Na-dependent Li transport, the red cells of two donors (R.B.G., B.S.) were used. The maximum rates of the Na-Li exchange in the cells of these donors were similar. Individual variations in the Na-Li transport were studied in the red cells of five male donors.

**Calculation of Fluxes**

Fluxes were calculated from the slope of the initial linear parts of graphs of ion concentrations in cells (influx) or medium (efflux) as a function of time as described in the previous paper (Pandey et al., 1978).

**RESULTS**

**Stimulation of Li Transport by Li and Na**

**Li Efflux**

External Na stimulation of Li efflux was measured in Li-loaded cells which contained intracellular Li and Na concentrations of 4.9 mM and 4.0 mM, respectively (Fig. 2). Increasing the extracellular Na concentration, by substituting for K, increased Li efflux from 0.14 mmol/(liter of cells × h) in an all-K medium to 0.33 mmol/(liter of cells × h) in an all Na medium. In the presence of phloretin no stimulation of Li efflux by external Na was observed. Neither choline, Mg, Ca, nor Rb could substitute for Na in stimulating Li efflux (see also Pandey et al., 1978). Li efflux increased and saturated with increasing external Na concentration (Fig. 2) yielding a straight line on a double reciprocal plot (Fig. 3) with a calculated maximum velocity (V_max) of 0.21 mmol/(liter of cells × h) for the Na-stimulated, phloretin-inhibited component and a half-maximum stimulation of Li efflux by 25 mM external Na.
Stimulation of Li efflux by intracellular Li concentration was measured at an external Na concentration of 144 mM which has been shown to saturate completely the external sites of the Li transport system with Na. Red cells were loaded with different concentrations of Li while adjusting internal Na to a low concentration (below 3 mM). Li efflux into the high Na media was a saturating function of intracellular Li concentration whereas Li efflux into an all-K medium or into a high Na medium supplemented with $2.5 \times 10^{-4}$ M phloretin was the same linear function of internal Li concentration (Fig. 4). The phloretin-sensitive portion of the Li efflux was thus equal to the external Na-stimulated Li efflux which followed a hyperbolic curve with increasing external Na concentration. On analysis of the external Na-stimulated Li efflux using a double reciprocal plot, the $V_{\text{max}}$ obtained was 0.22 mmol/(liter of cells x h) and half-maximal stimulation when the internal Li concentration was 0.5 mM (Fig. 5).

**Li influx** Externally-stimulated Li influx was measured in fresh, washed red cells. Li influx increased with increasing external Li concentration when Li was substituted for K, choline, or Mg in the medium. Li influx was a linear function of the external Li concentrations in the presence of phloretin under the same conditions (Fig. 6). Analysis of the phloretin-sensitive portion of the Li influx showed that it was also a linear function of external Na concentration (Fig. 7).
FIGURE 3. External Na-stimulated Li efflux is analyzed on a double-reciprocal (Lineweaver-Burk) plot. For loading and efflux conditions see the legend for Fig. 2. Half-maximum stimulation occurred at 25 mM external Na; maximum Li flux was 0.21 mmol/(liter of cells × h).

FIGURE 4. Stimulation of Li efflux by internal Li. Red cells were loaded with Li as described in Methods. Intracellular Na concentration was 2.1–2.2 mmol/liter of cells (designated mM on abscissa). Li efflux was measured either in 144 mM NaCl with and without phloretin (2.5 × 10^{-4} M) or in 144 mM KCl media, supplemented with 10^{-4} M ouabain and 16 mM Tris-HCl, pH 7.4 at 37°C. Hematocrit was 3%.
FIGURE 5. Stimulation of Na-dependent Li efflux by internal Li. The differences between Li efflux into a NaCl and into a KCl medium were measured as a function of internal Li. For leading and incubation conditions see legend for Fig. 4. Half-maximum stimulation occurred at 0.5 mmol/liter of cells (designated mM on abscissa) internal Li and the maximum rate of Na-stimulated Li efflux was 0.22 mmol/(liter of cells x h).

FIGURE 6. Stimulation of Li influx by external Li. Washed red cells (internal Na 7.8 mmol/liter of cells) were incubated in media with 0-16 LiCl substituted for KCl (144-128 mM). The media also contained $10^{-4}$ M ouabain and 16 mM Tris-HCl, pH 7.4. Li influx was measured at 37°C in the absence and in the presence of $2 \times 10^{-4}$ M phloretin. Hematocrit was 3%.
sensitive portion of the Li influx gave \( V_{\text{max}} \) of 0.15 mmol/(liter of cells \( \times h) \) and a half-maximal stimulation by 1.6 mM external Li (Fig. 7). The phloretin-sensitive Li influx was inhibited when 32 mM external Na was substituted for K, choline, or Mg in the medium. Analysis of the data on a double reciprocal plot showed competitive inhibition of Li influx by external Na, with an apparent inhibitory constant of 20 mM Na (Fig. 7). This concentration is approximately the same as that found for half-maximal stimulation of Li efflux by external Na (Fig. 3).

Stimulation of Li influx by internal Na was measured in red cells in which Na was substituted for internal K using the nystatin method. Cells with different internal Na concentration (0-115 mM Na, 140-25 mM K) were placed in isosmotic KCl or MgCl\(_2\) solutions containing 6.4 mM LiCl. Li influx increased along a hyperbolic curve as cellular Na increased (Fig. 8), whereas no stimulation of Li influx by internal Na was observed if phloretin was present in the medium. The graph of the data for the Na-stimulated (phloretin-sensitive) Li influx gave a straight line on a double reciprocal plot with a calculated \( V_{\text{max}} \) stimulation of Li influx by 9 mM internal Na (Fig. 9).

**Stimulation of Na Transport by Li**

**Stimulation of Na Efflux by Low Concentrations of External Li** To study Na movements through the Na-Li exchange system we measured the effect of external Li on Na efflux. \(^{22}\)Na efflux from red cells preloaded with tracer Na was significantly stimulated when external Li was substituted for K.
Below an external Li concentration of 16 mM, Na efflux was a saturable function of external Li concentration, with a calculated $V_{\text{max}}$ of 0.15 mmol/(liter of cells × h) and an apparent half-maximal stimulation by 1.4 mM external Li (Fig. 10).

**STIMULATION OF NA EFFLUX BY SUBSTITUTING HIGH CONCENTRATIONS OF EXTERNAL Li, CHOLINE, AND Mg FOR K** Above 16 mM external Li concentration, Na efflux increased only slightly until 80 mM Li was reached, then increased steeply as external Li was further increased to 145 mM and K was completely removed (Fig. 11). When external choline was substituted for K, Na efflux was independent of the choline concentration as long as it was below 80 mM. At external choline concentrations above 80 mM (external K reduced below 65 mM), Na efflux increased steeply (Fig. 11). Not shown in Fig. 11 is the important fact that Na efflux increased when KCl was replaced by MgCl$_2$ – sucrose (Cl concentration 160 mM) along the same curve as was observed with choline-Cl. Furosemide (1 mM) or phloretin (2.5 × 10$^{-6}$ M) inhibited Na efflux significantly in a KCl medium and blocked any increase in the Na efflux due to the changes in external medium. Thus, Na efflux from human red cells has a furosemide- (and phloretin-) sensitive component which is independent of the high affinity Na-Li countertransport system and which is reduced by increasing external K concentration. We also found that Na efflux into an all choline medium was stimulated by low concentration of external Li. The extent of this stimulation and the concentration of external Li which caused half-maximum stimulation in

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**Figure 8.** Stimulation of Li influx by internal Na. Red cells were prepared using the nystatin method containing different concentrations of Na. Li influx was measured in a medium containing 100 mM MgCl$_2$, 6.4 mM LiCl, 16 mM Tris-HCl, 10$^{-4}$ M ouabain, pH 7.4, at 37°C. Hematocrit was 3%. Internal Na concentration is in the units of mmol/liter of cells on the abscissa.
choline medium was similar to the values obtained in the KCl medium when Li stimulation of Na efflux was measured.

To examine the nature of Na efflux into high Li or choline we measured Na and K efflux and Li or choline ([14C]choline) influx in the same experiments (Table I). In both Li and choline media both Na and K efflux had a furosemide-sensitive component. In high Li medium the furosemide-sensitive Li influx was about the same as the sum of the furosemide-sensitive Na efflux and K efflux. In medium containing only Choline-Cl, there was no furosemide-sensitive choline influx comparable to the size of Na + K efflux. These results are consistent with the presence of a furosemide-sensitive transport system which can accomplish K-Na cotransport, Na-Na or Na-Li exchange, or net Na extrusion, and which is inhibited by external K (see discussion).

**Stoichiometry of Na-Li Exchange**

Using the information reported above, we designed a series of experiments to measure the stoichiometry of the Li-Na exchange transport system. 22Na efflux and Li influx were measured simultaneously in cells which were preloaded with 22Na and had slightly elevated intracellular Na concentrations (13-15 mmol/liter cells). The media for the flux determinations contained 10^-4 M ouabain, and either KCl or MgCl2 with and without 6.4 mM LiCl and with and without 10^-4 M phloretin. Both the phloretin sensitive and the external Li-stimulated Na effluxes were measured. The former was always larger than the latter because phloretin, like furosemide, partially inhibits Na efflux into solutions which contain neither Na nor Li (see, for example, Fig. 11). Apparently these
compounds inhibit components of ouabain-insensitive Na efflux which are not involved in Li-Na countertransport. Thus, the ratio of phloretin-sensitive Li influx to the phloretin-sensitive Na efflux was < 0.5. However, the mean value of the ratio of the phloretin sensitive Li influx to the external Li-stimulated Na efflux was 0.98 ± 0.07 (SEM, n = 13). No significant difference was observed between the ratios in KCl and MgCl₂ media. Thus, under these experimental conditions, 1:1 exchange of Li for Na occurred through this transportation.

**Variations among Individuals**

Inasmuch as preliminary experiments showed large individual variations in the rate of ouabain-insensitive Li transport, the studies on the kinetic parameters were done using the red cells of two donors (R.B.G., S.B.). To study the nature of the interindivdual variations in the Na-Li exchange system, we selected experimental conditions in which the maximum capacity of this system and its affinity for external Na could be studied simultaneously. According to Figs. 4 and 5, the internal site of the exchange system is expected to be about 90% saturated at 3.0 mM internal Li. Under such circumstances the Li efflux through this mechanism should depend primarily on the external Na concentrations. Therefore, we measured Li efflux from the red cells of five different male donors into media with different Na concentration (0-144 mM). Cellular Li

![Graph](image-url)
concentrations were adjusted by preincubation to 2.8-3.1 mM before the efflux experiments; internal Na concentrations after Li loading varied between 4.8 and 7.7 mM.

When red cells were incubated in Na-free (144 mM KCl) media, the Li efflux divided by the intracellular Li concentration (rate constant for the Li leak) was about the same (0.22 h⁻¹) in all subjects examined. The maximum Na-dependent Li efflux (calculated by subtracting the Li efflux into a KCl medium from the Li efflux into a 144 mM NaCl medium) varied between 0.14 and 0.37 mmol/(liter of cells × h). Fig. 12 shows the results obtained in the red cells of four male subjects for the Na stimulation of Li efflux. Although the maximum Na-

![Graph showing stimulation of Na efflux by external Li or choline.](image)

**Figure 11.** Stimulation of Na efflux by external Li or choline. Fresh red cells were loaded with tracer Na using nigericin. Intracellular Na concentration was 7.9 mmol/liter of cells. External LiCl or choline-Cl (0-144 mM) were substituted for equal concentrations of KCl. The media contained 10⁻⁴ M ouabain and 16 mM Tris-HCl, pH 7.4, at 37°C. Hematocrit was 3%.

dependent Li efflux varied over a 2.5-fold range, the apparent affinity for external Na in stimulating Li efflux was about the same in each case (25-28 mM). To assure that the maximum transport rate was measured, in donors with low transport rate we repeated the experiments by preloading the red cells to a greater internal Li concentration (3.8-4.0 mmol/liter of cells). The maximum transport rates for the Na-stimulated Li efflux were the same, excluding the possibility that the exchange system was not completely saturated in the earlier experiments when the red cell Li concentration was 2.8-3.1 mmol/liter of cells.

In the red cells of the same five donors we also investigated variations in the rate of bicarbonate-stimulated Li transport (Table II). In these experiments, we measured Li efflux from red cells containing 7-11 mmol Li/liter of cells into 160
mM KHCO₃, keeping the pH constant at 7.4 with a pH stat. The mean rate constant for Li efflux in these experiments was 0.15 h⁻¹ (range 0.132-0.162), a value about seven times greater than the value for the Li leak in the absence of bicarbonate.

**DISCUSSION**

The experiments in this paper quantitate the stimulation and inhibition of Na and Li fluxes by these ions on the internal and external surfaces of the human red cell membrane. The experiments were carried out in the presence of ouabain which blocks Li and Na movements through the Na-K pump (see Pandey et al., 1978), and in the absence of bicarbonate in the incubation medium inasmuch as LiCO₃ ion-pair formation provides an additional pathway for Li movements in red cells (Funder and Wieth, 1967; Wieth, 1970; Funder et al., 1978). The resulting data are consistent with the presence of a tightly coupled, one-for-one exchange of Na and Li ions in the red cell membrane. This system can promote Li-Na heteroexchange in both inward and outward directions. It can produce Na-Li counterflow, and thus uphill Li movement in the presence of an oppositely directed Na gradient (Haas et al., 1975; Duhm et al., 1976; Pandey et al., 1977). In the absence of Li, the countertransport system probably performs Na-Na exchange but we do not have unequivocal proof of this point. In the Na-Li exchange transport system, Na and Li appear to compete for a limited number of transport sites on the interior and exterior membrane surface. This view is supported by the observation that both cis-membrane Li and trans-membrane Na stimulate net Li flux from cis to trans solutions and show saturation kinetics. External sodium reduces the effective loading of the external site by lithium for influx (Fig. 7). The external side of the mechanism appears to have only sites which bind single ions inasmuch as external sodium at low concentrations did not stimulate Li influx, but rather only caused inhibition.

**TABLE I**

<table>
<thead>
<tr>
<th>Na, K, Li, AND CHOLINE TRANSPORT IN HUMAN RED CELLS</th>
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</thead>
<tbody>
<tr>
<td><strong>Efflux</strong></td>
</tr>
<tr>
<td>Na</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>LiCl (144 mM) medium + Furosemide</td>
</tr>
<tr>
<td>Furosemide-sensitive</td>
</tr>
<tr>
<td>Choline-Cl (144 mM) medium + Furosemide</td>
</tr>
<tr>
<td>Furosemide-sensitive</td>
</tr>
</tbody>
</table>

Washed red cells were incubated in the media indicated plus 10⁻⁴M ouabain, and 20 mM Tris-HCl, pH 7.4, at 37°C. The concentration of furosemide was 1 mM. Net Na, K, and Li fluxes were measured. For measuring choline influx the choline-Cl medium was supplemented with ³⁴Cl choline-Cl (ICN).
In that the stoichiometry is one-for-one, the internal side of the mechanism must also have one class of transport sites which bind only one ion at a time.

This qualitative description of the Na-Li exchange system can be translated into a quantitative model. We assume that the reactions between Li and Na and sites on the membrane surfaces are fast compared to the rate at which loaded sites are translocated between the membrane surfaces and, further, that empty sites cannot traverse the membrane. This model yields the following equation characterizing the relation between the Li flux through the Li-Na exchange system \( V_{Li} \) to the concentrations of Li and Na in the intra and extra cell fluid (\( Li_i, Na_i, Li_o, Na_o \), respectively). Five constants \( V_{max} \), the maximum transport rate; \( K_{Li}, K_{Na}, K'_{Li}, K'_{Na} \), the concentrations of Li and Na required to half-activate the system on the inside and the outside membrane surfaces) are sufficient.

\[
V_{Li} = \frac{V_{max}(K_{Li}Na_o - K'_{Li}Na_i)}{(1 + K_{Na}Na_o)(1 + K'_{Na}Na_i)}
\]

This model is adequate to describe the data which we have thus far gathered on this transport system in human red cells. Values for the several parameters are shown in Table III.

Kinetically these data may describe the operation of many molecular mechanisms and therefore cannot be used to distinguish between them. Among these
is a mobile carrier model, in which the mobility of the unloaded carrier is much smaller than that of either ion-carrier complex (Fig. 1). However, transport functions in red cells are now often ascribed to intrinsic proteins which span the membrane. A spanning membrane protein may also behave kinetically like a mobile carrier (Patlak, 1957). For example, the Li-Na exchange system could involve a conformation change which alters the access of a binding site from one side to the other side only if a Na or Li ion is combined with the binding site. Both the mobile carrier and spanning protein models described above are "consecutive" mechanisms. Equally consistent with the Li-Na kinetics of the exchange system is a simultaneous mechanism in which two sites, one on each side of the membrane, must be complexed with Na or Li before the conformational changes take place.

### Table II

<table>
<thead>
<tr>
<th>Donor</th>
<th>Internal Li</th>
<th>Efflux k</th>
<th>k⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mmol/liter of cells</td>
<td>mmol/liter of cells × h</td>
<td></td>
</tr>
<tr>
<td>J.K.A.</td>
<td>4.92</td>
<td>0.738</td>
<td>0.150</td>
</tr>
<tr>
<td>J.K.</td>
<td>7.34</td>
<td>1.058</td>
<td>0.144</td>
</tr>
<tr>
<td>O.F.</td>
<td>7.49</td>
<td>0.989</td>
<td>0.132</td>
</tr>
<tr>
<td>B.S.</td>
<td>7.21</td>
<td>1.168</td>
<td>0.162</td>
</tr>
<tr>
<td>R.B.G.</td>
<td>5.73</td>
<td>0.928</td>
<td>0.162</td>
</tr>
</tbody>
</table>

Cells were washed three times in 160 mM LiHCO₃ (pH 7.4) before loading. Loading was performed in 160 mM LiHCO₃ for 20 min at 37°C in a pH-stated chamber (pH 7.40). After loading, cells were washed three times in 160 mM KHCO₃ (pH 7.4). Efflux measurements were made in a pH-stated chamber containing 160 mM KHCO₃. CO₂ was blown over the cell suspension to maintain the pH at 7.40.

### Table III

<table>
<thead>
<tr>
<th>Inside Na⁺</th>
<th>Overade Li⁺</th>
<th>Kᵦ</th>
<th>Vₘₐₓ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>Li⁺</td>
<td>mM</td>
<td>mmol/liter of cells × h</td>
</tr>
<tr>
<td>9.0</td>
<td>0.5</td>
<td>25.0</td>
<td>1.5</td>
</tr>
</tbody>
</table>

The apparent affinities of the exchange system for Na and Li indicate that the internal and external sites prefer Li 15-18-fold over Na. Although this ratio is the same on both surfaces, the absolute values for Li and Na are threefold greater for the internal sites. These differences may be explained with identical sites on the two surfaces if we consider the greater density of negatively charged phospholipids (e.g., phosphatidylserine) at the internal face of the red cell membrane (Bretscher, 1972). The greater negative surface charge would increase the concentration of mobile positive ions near the transport site, thus increasing the apparent affinities of the site as measured from bulk solution concentrations. If phosphatidylserine contributes a charge density to the inner membrane surface which is approximately one electronic charge per $6 \times 10^{-6}$
cm² greater than that present on the outer membrane surface, the concentration of mobile cations would be three times greater on the inner than on the outer surface. This thin solution layer is many times thicker than the mean free path length of ions in 150 mM salt solutions. Therefore, the differences in the affinities on the inside and outside may perhaps be ascribed to the excess internal negative surface charge. The negative charges of the sialic acid groups on the external portion of glycoporin are much farther from the membrane surface and may not significantly influence the local distribution of ion charges at the transport sites.

The Na-Li exchange system described here has high affinity for Li, less for Na, and not yet detected affinity for any other monovalent or divalent cations. The experiments presented also yield evidence for the presence of a low affinity Li transport system in human red cells. Na efflux into high Li or choline media (Fig. 11) and Li influx in a high Li medium (Table I) show the presence of other ouabain-insensitive pathway(s) for Na and Li transport in human red cells. The magnitude of furosemide-sensitive Na efflux and Li influx in a 140 mM LiCl medium is much higher than the maximum capacity of the high affinity Na-Li exchange system, and involves a furosemide-sensitive component of K transport as well. This furosemide and phloretin-sensitive Na efflux is present in an alkaline medium when there is no comparable furosemide-sensitive choline influx (Table I). Hoffman and Kregenow (1966) described an ethacrynic acid-sensitive, external Na-stimulated Na extrusion from human red cells (Pump II). Sachs (1972) extended their studies by showing net, uphill extrusion of Na into a predominantly choline-Cl or MgCl₂-sucrose medium. This Na extrusion is inhibited by external K, furosemide, and ethacrynic acid. A K-Na cotransport producing net Na movement was reported by Wiley and Cooper (1974). The experiments of Martin (1972) suggest the presence of an alkali cation-choline countertransport, which has high affinity for choline, and much lower affinity for Na, Li, and K. These pathways may carry Li at high Li concentrations. However, their in vivo role under therapeutic conditions at plasma Li concentrations around 0.5-1.2 mM seems to be negligible. The same is true for Li transport through the ouabain-sensitive Na-K pump (Pandey et al., 1978).

The distribution of Li ions between red cells and the blood plasma is thus determined by the Na-Li exchange system, bicarbonate-stimulated Li transport, and the drug-insensitive leak. In the present experiments we observed a large individual variation in the maximum capacity of the Na-Li exchange system, also described by others (Duhm and Becker, Duhm et al., 1977c; Pandey et al., 1977). Here we report that the observed maximum rate of Li transport in a given experiment is a function of the saturation of the system by Na at the trans side and of the Li-Na competition on the cis side of the membrane. Studying the interindividual variations at fully saturated states of the exchange system we found about threefold differences in the maximum transport rate. Variations of this degree have not been reported for ouabain-sensitive Na-K transport or anion transport in normal red cells. The apparent affinity for external Na was the same in all the different donors' red cells. This variability in maximum capacity, so unusual in red cell membrane transport phenomena, may permit the further
investigation of a genetic basis for these differences (Dorus et al., 1975). The variability of the Na-dependent Li transport and the similar rate of the leak and the bicarbonate-stimulated Li transport in different individuals suggest that the genetic polymorphism observed for Li distribution in clinical studies is produced by differences in the Na-Li exchange system.

The present results for the kinetics of the Na-Li exchange system, in conjunction with the data for other Li transport pathways, provide the possibility of developing a quantitative model for Li distribution between red cells and plasma under different conditions (Gunn et al.).

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REFERENCES


