Cotransport of Lithium and Potassium in Human Red Cells

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ABSTRACT This paper reports the presence in human red cells of an additional ouabain-insensitive transport pathway for lithium ions, the Li-K cotransport. Several kinds of observations support this conclusion. Cells loaded to contain only K, Na, or Li do not exhibit furosemide-sensitive efflux. Simultaneous presence of K and Li on the same side of the membrane mutually stimulates furosemide-sensitive Li and K fluxes from that side. Cells loaded with both Na and Li exhibit no furosemide-sensitive Li efflux. Thus, Li can apparently replace Na but not K on the outward Na-K cotransport system in human red cells. Furthermore, LiO, like K0, inhibits outward Na-K cotransport. Additional proof for coupled Li-K cotransport is provided by the observation that an outwardly directed K electrochemical potential gradient can drive net outward Li movement against its gradient. There are several differences between Li-K cotransport and Li-Na countertransport. The cotransport system has an apparent affinity for Li that is about one-half that for Na and 30 times lower than the countertransport system. Furosemide and chloride replacement inhibit cotransport but do not affect countertransport. The PCMB loading procedure irreversibly inhibits countertransport but not cotransport. Furthermore, the two systems can apparently function at maximal rates simultaneously. Present evidence, then, indicates that the two pathways can be separated operationally as two different systems.

INTRODUCTION

Studies on the mechanism of lithium transport in human red cells have established that several systems can mediate the transfer of this cation across the cell membrane. In the presence of ouabain, Li can be transported by the phloretin-sensitive Na-Li countertransport system (Haas et al., 1975; Duhm et al., 1976; Pandey et al., 1978), and as LiCO3 ion pairs by the DIDS-sensitive anion-exchange system (Funder et al., 1978) and possibly by the choline exchange system (Martin, 1977). The ouabain-sensitive Na-K pump can also transfer lithium into the red cell and promote active Na efflux in the absence of a competitive effect from external potassium (Beauge and Del Campillo, 1978).
From inner sites, lithium can be transported outward and promote K influx through the pump in the absence of internal sodium (Dunham and Benyk, 1977).

Studies conducted by Pandey et al. (1978) and Sarkadi et al. (1978) have demonstrated that lithium can replace Na in the ouabain-insensitive Na-Na exchange system. This transport system has higher affinity for Li than for Na and displays marked interindividual differences in its maximum rate of transport. Measurements of the maximum rate of transport of Na-Li countertransport in patients with essential hypertension, as well as in members of their families, suggests that the transport system is, at least in part, genetically determined.

This paper is concerned with studies designed to investigate whether lithium can also be accepted by the Na-K cotransport mechanism. An inward cotransport of [Na + K] was demonstrated by Wiley and Cooper (1974) in human red cells. Garay et al. (1981) have studied the activation of outward cotransport in \( \mu \)-chloromercuribenzenesulfonate (PCMBS)-loaded cells by measuring ouabain-insensitive, furosemide-sensitive Na and K effluxes into medium that contains neither Na nor K.

Our experiments indicate that lithium can be accepted by the furosemide-sensitive [Na + K] cotransport system (Canessa et al., 1980b). Lithium can replace Na (but not K) in outward cotransport. We also show that an outward K gradient can drive net efflux of Li against its gradient. The [Li + K] cotransport can be distinguished from Li-Na countertransport by several experimental criteria. Our results are consistent with the presence in human red cells of two distinct ouabain-insensitive translocation systems for Na or Li ions rather than two modes of operation of a single ouabain-insensitive Na transport system.

**MATERIALS AND METHODS**

**Chemicals**

KCl, NaCl, LiCl, and MgCl\(_2\) were purchased from Mallinckrodt, Inc., St. Louis, MO. Tris, PCMBS, cysteine, adenine, and ouabain were purchased from Sigma Chemical Co., St. Louis, MO. Phloretin was obtained from ICN Nutritional Biochemicals Division, Cleveland, OH. Furosemide was a gift of Hoechst-Roussel Pharmaceuticals, Inc., Sommerville, NJ. Nystatin was from E. R. Squibb & Sons, Inc., Princeton, NJ. All the solutions were done in double-distilled water. Incubation media and washing solutions were adjusted to 296-300 mosmol.

**Preparation of Red Cells**

Human red blood cells were drawn from healthy donors into heparin. Plasma and buffy coat were removed after centrifugation for 10 min at 3,000 g at 4°C in a Sorvall RC-6B centrifuge (Dupont-Sorvall Instruments, Newtown, CT). The cells were washed three times with a solution containing 75 mM MgCl\(_2\), 95 mM sucrose, 10 mM Tris-MOPS, pH 7.4 at 4°C (WS). To determine dry weight, 100 µl of packed red cells was pipetted into a tared Corning glass pipette (0.5% precision; Corning Glass Works, Corning, NY), weighed, and dried in an oven until constant weight was
reached (Canessa et al., 1980a). The hemoglobin/liter cell and the cation composition of the initial cells were determined.

**Loading of Red Cells by the PCMBS Procedure**

Washed red cells were suspended (4% hematocrit) in solutions containing variable amounts of Li and K. Cation loading solutions contained (mM): 1 MgCl₂, 2.5 Tris-PO₄, pH 7.4 at 4°C, 150 [Li + K].

By trial and error we determined the relationship between the Li concentration of the loading solution and the expected concentration in the red cells in millimoles per liter cells. Freshly prepared PCMBS (0.02 mM) was added to the loading solutions (Garrahan and Rega, 1967). The cells were kept in the cold with gentle agitation. The loading solution was changed after 6 h and then left for 15 h. The cells were collected by centrifugation for 5 min at 3,000 g and the remaining loading solution was carefully removed.

The cells were incubated in a shaker bath for 1 h at 37°C in a recovery medium containing (mM): 2 adenine, 3 inosine, 4 cysteine, 10 glucose, 2.5 Tris-PO₄ (pH 7.4 at 37°C), and usually 75 LiCl and 75 KCl. The recovery solution contained 150 mM LiCl when high cellular concentrations of Li were required. Afterwards, the cells were spun and washed six times with washing solution (WSO) containing (mM): 75 MgCl₂, 95 sucrose, 10 Tris-MOPS, pH 7.4 at 4°C, and 0.1 ouabain. To prepare cells containing a single cation (Na, K, or Li) and for [Na + Li], the PCMBS treatment was carried out at 2% hematocrit for 40 h; the loading solution was replaced after 6 and 20 h.

**Nystatin Loading**

Washed red cells were suspended at ~50% hematocrit in a solution containing 130 mM NaCl and 32 mosmol sucrose. A mixture of air and CO₂ was used for titration of the cell suspension down to pH 6.8 at 22°C (Dalmark, 1975). The cells were washed twice in the same solution and the pH of the medium was checked.

Loading solutions contained 32 mosmol sucrose and the appropriate ionic composition, 1 mM Tris-EGTA, pH 7.4 at 4°C, and 30 µg/ml nystatin in methanol. Cells were suspended in these media at 2% hematocrit, at 4°C for 20 min. Afterwards, cells were washed four times at 30°C with nystatin-free solution containing 0.1 mM ouabain, 10 mM glucose, and 1 mM Tris-phosphate buffer pH 7.4. The cells were then washed six times with cold WSO for efflux measurements. In every experiment, hematocrit, hemoglobin/liter cell, cell water (wt/vol), and cell cation composition were determined before and after cation loading.

**Isotonic Li Loading**

Washed red cells were incubated at 20% hematocrit for 3 h at 37°C in a solution containing (mM): 150 LiCl, 10 Tris-MOPS, pH 7.4 at 37°C, and 10 glucose. Lithium was eliminated by six washes in WS and centrifugation for 2 min at 6,000 g.

**Determinations of Cations**

Red cell cations were measured by atomic absorption. Appropriate dilutions in double-distilled water (1:50–1:500) of the 50% cell suspension were made in triplicate. The ion concentration was calculated per milliliter of original cells measuring hematocrit and hemoglobin in the fresh and loaded cells.

Li concentration in Mg medium was determined using standard solutions contain-
ing 5–200 μM Li in 75 mM MgCl₂. Li concentration in 150 mM Na was measured with Li standards in H₂O. Na and K in Mg medium with standards in H₂O. When another cation was present in millimolar amounts, standards of similar composition were used for calibration of the atomic absorption spectrophotometer (model 5000; Perkin-Elmer Corp., Norwalk, CT).

**Cation Efflux Measurements**

The Na-free, K-free efflux media contained (mM): 75 MgCl₂, 85 sucrose, 0.1 ouabain, 10 Tris-MOPS, pH 7.4 at 37°C. A stock solution of furosemide (20 mM) was freshly prepared by titration with Tris-OH to pH 7.4. The complete solubility of furosemide was checked by measuring the optical density at 340 nm. 50% suspensions of washed, loaded cells were diluted to ~4% hematocrit in cold (4°C) flux media. Triplicate tubes containing 1.6 ml of the flux suspension were incubated for 25, 50, and 75 min at 37°C. To stop the reaction, tubes were transferred to 4°C for 2 min and then centrifuged for 7 min at 6,000 g. The supernatants were transferred with plastic syringes into plastic tubes for cation-content analysis in the atomic absorption spectrophotometer. The efflux was calculated from the slope relating the external cation concentration of the nine samples with time. The slope and its standard deviation were converted into flux units of millimoles per liter cell per hour using appropriate factors derived from the measured hematocrit of the incubation media. The furosemide-sensitive component of the flux was calculated from the difference between the fluxes measured with and without the inhibitor. No corrections for lysis were done because the optical density at 540 nm was time independent.

**Measurements of Net Cation Movement**

Red cells were loaded by the nystatin procedure and incubated at 4% hematocrit in media of variable ionic composition containing (mM): 0.1 ouabain, 10 Tris-MOPS, pH 7.4 at 37°C, and 10 glucose. Quadruplicate samples were taken at 5 min and 5 h after incubation at 37°C. After spinning for supernatant separation, the cells were washed 4 times with cold WSO and lysed in appropriate volumes of Acationox (0.02%; American Scientific Products, McGaw Park, IL) for hemoglobin and electrolytes measurements.

**Chloride Replacement by Nitrate**

Red cells were loaded for measurements of the maximal rate of [Na + K] cotransport in a solution containing (mM): 120 NaCl, 30 KCl, 1 MgCl₂, 2.5 Tris-PO₄, pH 7.4, and 0.02 PCMBs. The cells were recovered after PCMBs treatment as previously described. The pellet was separated into two fractions, control and chloride free. Chloride was removed afterwards by two washes and incubation for 30 min in a solution containing (mM): 75 NaNO₃, 75 KNO₃, 1 Mg(NO₃)₂, and 2.5 Na phosphate, pH 7.4. Furosemide-sensitive Na and K effluxes were measured into a medium containing (mM): 75 Mg(NO₃)₂, 85 sucrose, 0.1 ouabain, 10 glucose, 10 Tris-MOPS, pH 7.4 at 37°C.

The red cells for measurements of the maximal rate of Li-Na countertransport were loaded for 2 h in 150 mM LiCl, 10 mM glucose, 10 mM Tris-MOPS, pH 7.4 at 37°C. Afterwards, the cells were divided into two batches. In the first batch, cells were incubated for 30 min in 150 mM LiNO₃, 10 mM glucose, 10 mM Tris-NO₃, pH 7.4 at 37°C. After two washes, the cells were incubated again in LiNO₃ loading solution for 30 min. The cells were washed six times with Mg(NO₃)₂-sucrose solution. Li efflux was measured into a medium containing (mM): (a) 75 Mg(NO₃)₂, 85 sucrose, 10
glucose, 10 Tris-MOPS, pH 7.4 at 37°C, and 0.1 ouabain; and (b) 150 NaNO₃, 10 glucose, 10 Tris-MOPS, and 0.1 ouabain. In the second batch, the LiCl loading solution was used instead of the LiNO₃ loading solution. The cells were washed six times with WSO. Li efflux was measured into a medium containing (mM): (a) 75 MgCl₂, 85 sucrose, 10 Tris-MOPS, pH 7.4 at 37°C, 0.1 ouabain, 10 glucose; and (b) 150 NaCl to replace the MgCl₂ and sucrose.

**RESULTS**

*Efflux from Cells Containing a Single Cation Is Furosemide Insensitive*

As an attempt to characterize the outward Na–K cotransport, Garay et al. (1980a, 1981) studied the effect of furosemide on the outward movement of Na and K. The stoichiometry of the furosemide-sensitive Na and K efflux was 1:1. The maximal activation of cotransport was approached over 25 mmol of Na per liter of cells.

**TABLE I**

| Cellular composition | Cation efflux potassium | Sodium | Lithium |
|----------------------|-------------------------|--------|---------|
| K                    | Na                      | Li      | Without furosemide | With furosemide | Without furosemide | With furosemide |
| mmol/liter cells     | mmol/l cells/h          |        |                      |                 |                     |                |
| PCMBS loading         |                         |        |                      |                 |                     |                |
| 99.0                 | 0.8                     | 2.30±0.6 | 2.40±0.5             | —                | —                    | —               |
| 2.0                  | 100.0                   | —       | —                    | 2.80±0.2         | 2.61±0.2             | —               |
| 2.5                  | 2.7                     | 118     | —                    | 3.30±0.4         | 3.50±0.4             | —               |
| Nystatin loading (n=3)|                         |        |                      |                 |                     |                |
| 101.5                | 0.1                     | 1.8±0.7 | 1.9±0.7              | —                | —                    | —               |
| 0.8                  | 105.0                   | —       | —                    | 1.8±0.2          | 2.0±0.3              | —               |
| 0.5                  | 0.8                     | 111     | —                    | 1.8±0.5          | 2.0±0.8              | —               |

Cation efflux was measured by incubating the loaded cells at 3% hematocrit in (mM): 75 MgCl₂, 85 sucrose, 10 glucose; 0.1 ouabain, 10 Tris-MOPS, pH 7.4 at 37°C, with and without 1 mM furosemide. 

n = number of different donors. Mean values ± SE. 

PCMBS experiments were n = 5 for K cells, n = 1 for Na cells, and n = 4 for Li cells.

Table I shows ouabain-insensitive cation efflux into Mg medium from red cells loaded with only one cation such as Na, K, or Li by the PCMBS and nystatin loading procedures. The outward movement of Na from Na-loaded cells, of K from K-loaded cells, and of Li from Li-loaded cells was found to be furosemide insensitive.

We found it difficult to obtain Na-loaded cells and Li-loaded cells with cellular K below 2 mmol/liter cell and normal cell volume by means of the usual PCMBS procedure. The use of higher PCMBS concentrations and longer incubation periods could produce K-free cells with increased K permeability. In contrast, the nystatin loading procedure permits preparation of K-
free cells with less of an increase in K permeability. Moreover, an accurate prediction of cell volume is produced by adjusting the osmotic pressure contribution of sucrose (Dalmark, 1975).

**Lithium Can Replace Na in a [Na + K] Cotransport Mechanism**

Fig. 1 shows furosemide-sensitive cation efflux from experiments in red cells loaded by the nystatin procedure with three different cation pairs [Na + K], [Li + K], and [Na + Li]. Na and Li efflux were furosemide sensitive in cells containing K. The furosemide-sensitive Li efflux from cells loaded with [Li + K] was slightly lower than the furosemide-sensitive Na efflux from [Na + K] loaded cells. The stoichiometric ratio between furosemide-sensitive K and Li efflux in this experiment was 1:1. It can also be seen in Fig. 1 that in red cells loaded with [Na + Li], Na efflux was furosemide insensitive. Table II shows simultaneous measurements of Li-K and Na-K cotransport in cells of five different donors loaded by the nystatin procedure. Cotransport of lithium is slightly lower than that of Na in cells containing ~40 mmol/liter K. The ratio of furosemide-sensitive Na to furosemide-sensitive K efflux was 1.0, while the furosemide-sensitive Li/K was 1.3, not significantly different.

**Dependence of Li-K Cotransport on Cellular Lithium Concentration**

To investigate the dependence of the outward Li-K cotransport system on the internal lithium concentration, red cells were loaded with increasing amounts...
of Li (at the expense of K) by the PCMBS method. Li and K effluxes were measured into a Mg medium containing 0.1 mM ouabain with and without 1 mM furosemide.

Li efflux was observed to be greater in the absence than in the presence of furosemide over the range of Li concentrations between 8 and 100 mmol/liter cells (Fig. 2). Over this range, the relation between furosemide-insensitive efflux and Li concentration is linear. The rate constant for Li efflux in the presence of furosemide was 0.011 h⁻¹ (Fig. 2). The ouabain-insensitive, furosemide-resistant K efflux was also a linear function of internal K yielding a rate constant of 0.013 h⁻¹ (Table III).

The magnitude of the furosemide-sensitive Li efflux increased as cell Li concentration increased and K concentration decreased (Fig. 2). Furosemide-sensitive K efflux also increased when cell Li concentration increased from 0 to 60 mmol/liter and cell K concentration decreased from 100 to 40 mmol/liter. In red cells of one donor, similar results were obtained with nystatin and PCMBS loading procedures.

Table II summarizes mean values of the maximum furosemide-sensitive efflux of Li and K taken from experiments of the type shown in Fig. 2 on 12 different subjects. Also shown in the table are the concentrations of cell Li and K at which the furosemide-sensitive effluxes were maximal. Cellular Li concentrations between 50 and 70 mmol/liter cell were required for saturation of the furosemide-sensitive Li efflux. The ratio between furosemide-sensitive Li/K effluxes in PCMBS-treated cells was 1.6 ± 0.5 (n = 11) and 1.3 ± 0.3 in nystatin-loaded cells (n = 5), neither being significantly different from another (Table II).

**Table II**

| Cellular composition | Furosemide-sensitive efflux | Ratio |
|----------------------|-----------------------------|-------|
| Na       | K     | Li     | Na | K | Li | Na/K | Li/K |
| mmol/liter cells | mmol/liter cells/h |       | mmol/liter cells/h |
| 57       | 40    | —      | 0.56±0.06 | 0.59±0.05 | —   | 1.04±0.11 | —     |
| —       | 36    | 58     | —   | 0.34±0.04 | 0.42±0.05 | — | 1.34±0.30 |

Mean ± SE.

Simultaneous measurements in four different donors. All fluxes were measured into Mg-sucrose medium containing 0.1 mM ouabain and/or 1 mM furosemide, as indicated in Methods.

Table III summarizes mean values of the maximum furosemide-sensitive efflux of Li and K taken from experiments of the type shown in Fig. 2 on 12 different subjects. Also shown in the table are the concentrations of cell Li and K at which the furosemide-sensitive effluxes were maximal. Cellular Li concentrations between 50 and 70 mmol/liter cell were required for saturation of the furosemide-sensitive Li efflux. The ratio between furosemide-sensitive Li/K effluxes in PCMBS-treated cells was 1.6 ± 0.5 (n = 11) and 1.3 ± 0.3 in nystatin-loaded cells (n = 5), neither being significantly different from another (Table II).

**Lithium Can Be Driven against Its Electrochemical Gradient by a K Gradient**

Table IV shows that net lithium transport against its electrochemical gradient can be promoted by an outward K gradient. Red cells were loaded by the nystatin procedure to contain grossly equal amounts of [Li + K] and [Li + Na]. Net cation movement was determined after 6 h of incubation in ouabain-containing media with and without furosemide. A net loss of cellular lithium took place from the cells containing [Li + K] into a medium containing 90
mM K. A fraction of this Li lost is furosemide sensitive (0.26 mmol/liter cells/h). The inward K gradient moved more K into these cells in the presence of furosemide. Therefore, the outward Li gradient translocated 0.44 mmol/liter cell/h of K against a gradient $K_i/K_o = 82/90$ (mmol/kg water).

When an outward K gradient ($K_i/K_o = 82/0$) was set up for the [Li + K] loaded cells, 0.48 mmol/liter cells/h of lithium were transported by a furosemide-sensitive mechanism, concomitantly with a similar furosemide-sensitive loss of K.

In cells containing [Li + Na], Na was not transported uphill with an outward lithium gradient; there was net Na gain inhibited by furosemide from 0.6 to 0.16 mmol/liter cells/h. The outward Na gradient did not move
lithium uphill because the small fraction of the furosemide-sensitive Li gain was not statistically significant. The downhill movement of lithium from the [Li + Na] cells into Na medium takes place via a furosemide-insensitive pathway that may comprise Li/Na exchange and a diffusional pathway. We do not have an explanation for the inhibition by furosemide of the Na gain into [Na + Li] cells.

### Table III

| Cellular Composition | Furosemide-sensitive efflux | Furosemide-resistant rate constant |
|----------------------|-----------------------------|-----------------------------------|
| Li       | K   | Na    | Lithium | Potassium | Lithium | Potassium |
| mmol/liter cells | mmol (liter cells/h)⁻¹ | h⁻¹ |
| Mean    | 62  | 38    | 2       | 0.32      | 0.20    | 0.015     | 0.013     |
| SD      | 10  | 7.4   | 1       | 0.11      | 0.06    | 0.004     | 0.004     |

Number of experiments: 12.

### Table IV

**NET UPHILL TRANSPORT OF LITHIUM DRIVEN BY AN OUTWARD K GRADIENT IN HUMAN RED CELLS**

| Cellular composition | Medium | Lithium | ΔF | Potassium | ΔF | Sodium | ΔF |
|----------------------|--------|---------|----|-----------|----|--------|----|
| Li       | K   | Na    | mmol/kg cell water | mmol/liter cells/h |
| 75       | 82  | 0.3   | 90 mM K + furosemide     | -0.94      | +0.18   |
| 75       | 82  | 0.3   | 90 mM Li + furosemide    | -0.70      | -0.26±0.18 | +0.62 | -0.44±0.20 |
| 65       | 0.1 | 70    | 90 mM Na + furosemide    | -0.74      | +0.60   |
| 65       | 0.1 | 70    | 90 mM Li + furosemide    | +0.56      | +0.16 | +0.44±0.10 |
|           |      |       | +0.36                  | +0.20±0.11 | -0.60 | 0     |

Cells were loaded by the nystatin procedure, washed, and suspended in Mg-sucrose solution. The external medium also contained (mM): 60 choline, 10 Tris-MOPS, pH 7.4 at 37°C, 10 glucose, 0.1 ouabain, with and without 1 furosemide. Hematocrit: 4%. Net fluxes were calculated from a 6-h incubation period. n = three experiments.

**Li⁺-Na⁺ Countertransport Is Furosemide Resistant**

To investigate whether the Li-Na exchange pathway is a mode of operation of the Li-K cotransport system, we have studied the effect of furosemide on the Li⁺/Na⁺ and the Na⁺/Li⁺ exchange.

Fig. 3 shows the effect of furosemide on the Li⁺/Na⁺ exchange system. Red cells were loaded to contain 10 mmol/liter cells of lithium at which the exchange system approaches maximal rate. The Na⁺-stimulated component
of lithium efflux was 0.27 mmol/liter cells/h in this experiment. Phloretin (0.2 mM) completely inhibited the Na<sub>e</sub>-dependent lithium efflux. However, 1 mM furosemide did not inhibit Na<sub>e</sub>-Li<sub>i</sub> countertransport, but did inhibit Li efflux into Mg medium. Table V shows the results obtained in 10 experiments with different donors. Notice that furosemide slightly inhibits Li efflux both into Mg medium and into Na medium but did not significantly inhibit the Na<sub>e</sub>-stimulated Li efflux (−0.03 ± 0.04, P < 0.1).

We also investigated the effect of furosemide on Na<sub>e</sub>-stimulated Li efflux at several external Na<sub>e</sub> concentrations (data not shown). Furosemide produced a slight (~10%) inhibition of the transport system at all concentrations tested (5, 10, 25, 50, 75, and 100 mM Na<sub>e</sub>). Cotransport and countertransport have different sensitivity to furosemide. Cotransport is maximally inhibited by 0.5 mM furosemide, whereas countertransport can be inhibited up to 50% by 5 mM furosemide (data not shown). Similarly, 10<sup>−8</sup> M bumetamide maximally inhibited cotransport but only 10% of countertransport. This result indicates that lithium translocation by a phloretin-sensitive countertransport system is largely furosemide resistant.

Table VI shows the effect of furosemide on Li efflux from cells loaded to achieve the maximal rate of Li<sub>i</sub>/Na<sub>e</sub> countertransport and Li-K outward cotransport. The Na<sub>e</sub>-stimulated Li efflux (Li<sub>i</sub>-Na<sub>e</sub> countertransport) is of...
TABLE V

EFFECT OF FUROSEMIDE ON SODIUM-STIMULATED LITHIUM EFLUX FROM HUMAN RED CELLS

| Experiment | Mg medium | Na medium | Countertransport |
|------------|-----------|-----------|------------------|
|             | Alone     | With furosemide | Alone | With furosemide | Alone | With furosemide |
| 80-1        | 0.20±0.02 | 0.15±0.02 | 0.67±0.08 | 0.52±0.01 | 0.47 | 0.37 |
| 80-3        | 0.18±0.01 | 0.12±0.01 | 0.39±0.08 | 0.33±0.01 | 0.21 | 0.21 |
| 80-4        | 0.19±0.03 | 0.14±0.006 | 0.48±0.007 | 0.38±0.01 | 0.29 | 0.24 |
| 80-5        | 0.52±0.02 | 0.34±0.02 | 0.79±0.03 | 0.61±0.02 | 0.27 | 0.27 |
| 20          | 0.27±0.02 | 0.21±0.02 | 0.51±0.06 | 0.43±0.06 | 0.24 | 0.22 |
| 37          | 0.24±0.01 | 0.20±0.009 | 0.37±0.01 | 0.32±0.017 | 0.13 | 0.12 |
| 46          | 0.13±0.005 | 0.08±0.001 | 0.62±0.01 | 0.50±0.01 | 0.49 | 0.42 |
| 48          | 0.10±0.005 | 0.07±0.001 | 0.31±0.01 | 0.26±0.01 | 0.21 | 0.19 |
| 51          | 0.16±0.006 | 0.17±0.006 | 0.36±0.07 | 0.30±0.01 | 0.20 | 0.13 |
| 82          | 0.14±0.003 | 0.10±0.003 | 0.36±0.006 | 0.27±0.007 | 0.21 | 0.17 |
| Mean        | 0.21       | 0.16       | 0.49       | 0.39       | 0.27 | 0.24 |
| ± SE        | 0.03       | 0.025      | 0.05       | 0.037      | 0.03 | 0.03 |

All fluxes are in mmol/liter cells/h. Lithium was loaded up to 9 mmol/liter cells by incubation in LiCl. The Mg and Na media contained 0.1 mM ouabain with and without 1 mM furosemide.

TABLE VI

EFFECT OF FUROSEMIDE ON LITHIUM COUNTERTRANSPORT AND COTRANSPORT

| Cellular concentration | Li mmol/liter cells | K mmol/liter cells | Furosemide | Mg Medium mmol/liter cells/h | Na Medium mmol/liter cells/h | ΔNa mmol/liter cells/h |
|------------------------|---------------------|--------------------|------------|-----------------------------|-----------------------------|------------------------|
|                        | 9.5                 | 93                 | −          | 0.12±0.01                   | 0.45±0.01                   | 0.33±0.01              |
|                        |                     |                    | +          | 0.07±0.01                   | 0.37±0.01                   | 0.30±0.01              |
|                        |                     |                    | Δ          | 0.05±0.014                  | 0.08±0.014                  |                       |
|                        | 64                  | 39                 | −          | 1.11±0.06                   | 1.61±0.07                   | 0.50±0.09              |
|                        |                     |                    | +          | 0.83±0.06                   | 1.20±0.10                   | 0.40±0.12              |
|                        |                     |                    | Δ          | 0.27±0.08                   | 0.41±0.12                   |                       |

The red cells from the same donor were loaded by the nystatin procedure. Efflux media contained 0.1 mM ouabain, 10 mM glucose, 10 mM Tris-MOPS, pH 7.4 at 37°C. Mg media contained 85 mM sucrose and 75 mM MgCl₂. Na media contained 4 mM MgCl₂. (n = 3 experiments in three different donors.)

In both types of cells, the ΔNa in the absence of furosemide (0.50±0.33 = 0.17 ± 0.090, P > 0.1) and in the presence of furosemide (0.30±0.40 = –0.10 ± 0.12, P > 0.1) are not statistically different.

In similar magnitude in both types of cells in the presence and absence of furosemide. Furthermore, in cells loaded to contain 64 mmol/liter Li and 39 mmol/liter K (optimal for Li-K cotransport), furosemide inhibits both in the presence and in the absence of external Na. Thus, maximum rates of outward
movement of Li by Li-Na countertransport and Li-K cotransport can apparently occur simultaneously.

**External Li Inhibits Both Furosemide-sensitive Na Efflux and K Efflux**

Table VII shows the effect of external Li on outward Na-K cotransport. The cells were loaded by nystatin with [Na + K] to achieve the maximal rate of cotransport. The furosemide-sensitive Na and K effluxes were inhibited by external Li. The table also shows the stimulation of furosemide-insensitive Na (but not K) efflux by external Li. This point is made more clearly in Table VIII, where some of the same data are shown but with the external Li-stimulated, furosemide-insensitive Na efflux shown explicitly. Table VIII also shows that Li influx approximately equals the Li-stimulated Na efflux under these conditions. Furthermore, Na-stimulated Li efflux in the red cells of this subject was 0.25 mmol/liter cells/h, not significantly different from Li-stimulated Na efflux. Furthermore, Table VIII also shows that phloretin-sensitive Li-stimulated Na efflux, and the phloretin-sensitive Li influx, were approximately equal in this experiment done in the presence of furosemide. These data indicate that phloretin, but not furosemide, inhibits the Li/Na exchange pathway.

We also investigated the effect of phloretin on the Na-K cotransport. Fig. 4 shows the effect of 0.2 mM phloretin on the ouabain-insensitive Na and K effluxes. Red cells were loaded with [Na + K] by the nystatin procedure to achieve maximal activation of cotransport. The ouabain-insensitive Na and K effluxes were inhibited by 1 mM furosemide but not by 0.2 mM phloretin. Na efflux was not significantly reduced, and K efflux was enhanced by phloretin. The effects of phloretin were different on red cells from different donors. In some, Na efflux was slightly inhibited but K efflux was always increased. Phloretin also produced a variable inhibition of Li efflux into Mg medium (Fig. 3), which we interpreted as inhibition of Li-K cotransport.

**Table VII**

| Medium     | Furosemide | Na Efflux | K Efflux |  
|------------|------------|-----------|----------|  
| Mg-sucrose | -          | 1.77±0.09 | 1.31±0.09 |  
| +          | 1.23±0.08  | 0.68±0.06 |          |  
| Δ          | -0.54±0.15 | -0.63±0.10|          |  
| 20 mM Li   | -          | 1.65±0.08 | 1.00±0.08|  
| +          | 1.60±0.10  | 0.79±0.07 |          |  
| Δ          | -0.05±0.12 | -0.21±0.11|          |  

Red cells were loaded by the nystatin procedure to contain (mmol/liter cells) 64 Na and 44 K. (n = 3 experiments in three different donors.)
Table IX shows the effect of PCMBS loading procedures on lithium transported by the countertransport system. In the control cells, the loading was achieved by incubation at 37°C in (mM): 150 LiCl, 10 Tris-MOPS, pH 7.4, 10 glucose solution. It can be seen that the Li–Na countertransport does not recover after incubation in cysteine-containing media following the long incubation in PCMBS solutions. The Li–Na countertransport was inhibited by 60% in 12 different donors (Table IX). Marked changes in the affinity for internal lithium were found in PCMBS-loaded cells.

The Li–Na countertransport, measured by the nystatin loading procedure, gave values equal to those obtained by the isotonic Li loading procedure (Canessa and Tosteson, 1979).

### Table VIII

| Cells | Medium | Na efflux | Li influx | Li efflux |
|-------|--------|-----------|-----------|-----------|
| 66 Na, 44 K | Mg-sucrose | 1.23±0.08 | — | — |
| 20 mM Lithium | 1.60±0.10 | 0.41±0.01 | — |
| ΔLi | 0.37±0.13 | — | — |
| 10 Li, 90 K | Mg-sucrose | — | — | 0.07±0.01 |
| 150 mM Na | — | — | 0.32±0.01 |
| ΔNa | — | — | 0.25±0.014 |

Red cells of the same donor in Table VIII were loaded by the nystatin procedure, washed, and suspended in MgCl₂-sucrose-ouabain solution. All flux media contained 0.1 mM ouabain and 1 mM furosemide. Na medium contained 4 mM MgCl₂. Phloretin-sensitive Li influx was 0.3 ± 0.02 mmol/liter cells/h.

### Chloride Ions Are Required for Cotransport But Not for Countertransport

Table X shows the effect of the replacement of chloride by nitrate on the Na⁺-stimulated Li efflux and in Na-K cotransport in human red cells. It can be seen that the lithium countertransport operates as well in nitrate-containing media as in chloride-containing media. On the contrary, furosemide-sensitive Na-K cotransport is inhibited in the presence of nitrate as anion.

### Discussion

Lithium, the smallest group I cation, can substitute for Na in several passive transport systems such as the Na channel of the axonal membrane (Moore et al., 1966; Hille, 1972), the amiloride-sensitive Na entry step into epithelial cells (Herrera, 1972; Reinach et al., 1975; Nagel, 1977), the bicarbonate anion exchange (Funder et al., 1978), and the countertransport system of red cells (Pandey et al., 1978; Duhm et al., 1976).

In this paper, we demonstrate that Li can also replace Na in the outward
Na-K cotransport system in human red cells. Three lines of evidence support this conclusion.

First, when K and Li are simultaneously present on the same side of the membrane, they mutually stimulate furosemide-sensitive fluxes from that side (cis stimulation). Thus, in red cells loaded to contain only one cation inside (either K, Na, or Li), the efflux of K, Na, and Li into Mg medium was not furosemide sensitive (no cotransport) (Table I). By contrast, lithium efflux from cells containing [Li + K] was found to be furosemide sensitive. Two different procedures were used to adjust the cellular lithium and potassium concentration: the PCMBS loading procedure and the nystatin loading procedure. Similar results were obtained with both methods. The maximal activation of furosemide-sensitive Li efflux from cells loaded with [Li + K] was observed between 50–70 mmol/liter cell of Li. In comparison, in red cells loaded with [Na + K] the maximal activation of furosemide-sensitive Na efflux into Mg media was observed between 25 and 80 mmol/liter cell of Na (Garay et al., 1981). These results suggest that the Na sites in the outward cotransport system may have a higher affinity for Na than Li.

Lithium does not seem to replace K in the outward cotransport mechanism. In red cells loaded with [Na + Li], lithium efflux was not inhibited by furosemide. These results agree with experiments on inward [Na + K] cotransport reported by Wiley and Cooper (1974). They showed that 150 mM external K, but not 150 mM LiCl, conferred furosemide sensitivity to Na.
influx from 10 mM Na. However, 150 mM external lithium promoted furosemide-sensitive K influx equivalent to half the flux stimulated by 150 mM Na.

The second line of evidence for Li participation in the Na-K cotransport

**Table IX**

**INHIBITION BY THE PCMBS LOADING PROCEDURE OF THE Li-Na COUNTERTRANSPORT**

| Li efflux | Mg medium | Na medium | Counter-transport | Percent inhibition |
|-----------|-----------|-----------|-------------------|-------------------|
| mmol/liter cells/h | | | | |
| Loading in | 0.17±0.018 | 0.49±0.03 | 0.32±0.05 | 60±3 |
| 150 mM LiCl | | | | |
| PCMBS | 0.20±0.013 | 0.33±0.023 | 0.13±0.03 | |

Mean ± SE.

n = 12 different donors.

Li concentration was 8 mmol/liter cells.

**Table X**

**EFFECT OF CHLORIDE REPLACEMENT ON COUNTERTRANSPORT AND COTRANSPORT**

| [Na-K] cotransport | Na efflux | K efflux |
|--------------------|-----------|----------|
| mmol/liter cells/h | | |
| Chloride | 1.02±0.08 | 0.65±0.04 |
| Nitrate | 0.27±0.02 | 0.23±0.03 |

| [Li-Na] countertransport | Na-stimulated Li efflux |
|--------------------------|--------------------------|
| mmol/liter cells/h | | |
| Chloride | 0.21±0.06 |
| Nitrate | 0.22±0.08 |

Cotransport cells were loaded by the PCMBS procedure to contain approximately equal amounts of Na and K and washed with WSO. Na and K effluxes were measured into medium containing (mM): 75 MgCl₂, 85 sucrose, 0.1 ouabain, 10 glucose, 10 Tris-MOPS, pH 7.4 at 37°C, hematocrit 4%. Cotransport cells were loaded by incubation in isotonic LiCl as indicated in Methods. The cells contained (mmol/liter cells) 10 Li, 5 Na, and 86 K. After washing, Na-stimulated Li efflux was determined by measuring Li efflux into Mg and Na medium as indicated in Methods. The hematocrit of the flux media was 4%. Four experiments were done in three different donors.

system is the capacity of Li to produce trans inhibition. Thus, external Li inhibits furosemide-sensitive effluxes of Na and K (Table VII). External K also inhibits outward Na-K cotransport (Wiley and Cooper, 1974; Sachs, 1971; Garay et al., 1981).
Additional proof for coupled Li-K cotransport rather than cis activation of furosemide-sensitive fluxes is provided by the demonstration of net movement of both cations. An outwardly directed Li electrochemical potential gradient can drive net outward K movement, whereas an outwardly directed K gradient can drive net outward Li movement (Table IV). By contrast, an outwardly directed Na gradient cannot drive net outward Li movement (Table IV).

Table XI summarizes several properties of the countertransport and cotransport systems. Several criteria of discrimination between these two pathways of lithium translocation are given in this paper. The two Na transport systems have different kinetic parameters. Lithium has a 50-times-higher apparent

| TABLE XI |
| PROPERTIES OF Na COUNTERTRANSPORT AND Na COTRANSPORT SYSTEMS OF HUMAN RED CELLS |

| Countertransport | Cotransport |
|------------------|-------------|
| Countercurrent   | Cotransport |
| Li > Na          | Na > Li     |
| k<sub>i</sub>/<sub>1/2</sub> = 0.5 mM | k<sub>i</sub>/<sub>1/2</sub> = >25 mM |
| k<sub>i</sub>/<sub>1/2</sub> = 9 mM | k<sub>i</sub>/<sub>1/2</sub> = 13 mM |

1. Cation side effects
   - Trans-stimulated by Na or Li
   - Cis-stimulated by K

2. "Apparent" affinity for internal sites
   - Li > Na
   - Na > Li
   - k<sub>i</sub>/<sub>1/2</sub> = 0.5 mM
   - k<sub>i</sub>/<sub>1/2</sub> = >25 mM
   - k<sub>i</sub>/<sub>1/2</sub> = 9 mM
   - k<sub>i</sub>/<sub>1/2</sub> = 13 mM

3. Effect of inhibitors
   - Phloretin: Inhibits
   - Furosemide: Inhibits
   - PCMBS loading: Inhibits

4. Effect of changes in cell volume
   - Swelling: Insensitive
   - Sensitive

5. Inter-individual variance
   - V<sub>max</sub>
   - K<sub>1/2</sub> and V<sub>max</sub>

6. Chloride replacement by nitrate
   - Insensitive
   - Inhibits

7. Stoichiometry of in-out movements
   - 1 (Na or Li):1 (Na or Li)
   - 1 (Na or Li):1 (K)
These results are in agreement with studies by Motais and Sola (1973) and Duhm and Becker (1979) and Becker and Duhm (1979). Sachs (1971) also showed in fresh human erythrocytes that ouabain-insensitive Na efflux into a Na-free medium was stimulated by external Na in the absence and presence of 1 mM furosemide. These results suggest that Li-Na, Na-Li, and Na-Na exchange are all furosemide insensitive.

Another difference between cotransport and countertransport was found after loading the red cells by the PCMBS technique. The cotransport system recovers completely from the PCMBS inhibition by incubation in a cysteine medium (Garay et al., 1981). However, the Li-Na countertransport was inhibited by this procedure. The effect of prolonged incubation in PCMBS containing media might be related to the incomplete desorption of the inhibitor after the recovery step as described by Sutherland et al. (1967).

We have also shown that the two systems can apparently operate simultaneously. The Na-stimulated Li efflux is the same at low and high internal Li concentrations (Table VI). At the lower concentrations, cotransport is minimal, whereas at the higher concentrations it is maximal. Furthermore, the furosemide-sensitive K efflux was slightly higher in the presence (countertransport active) than in the absence (countertransport inactive) of external Na. Both transport systems can be differentiated by their independent responses to changes in cell volume. Cell swelling substantially inhibited the cotransport but did not affect the countertransport system (Adragna et al., 1980).

One interesting property that both of these transport systems share is that the maximum rates vary from individual to individual in the human population (Wiley, 1977; Pandey et al., 1979; Garay et al., 1980b). These differences are, at least in part, inherited (Pandey et al., 1977; Canessa et al., 1980a). The differences may provide insight into the pathogenesis and genetic transmission of disorders associated with these transport systems such as hereditary anemia (Wiley, 1977), mania (Pandey et al., 1977), and hypertension (Canessa et al., 1980a).

Finally, the replacement of chloride ion by nitrate completely blocked the Na-K cotransport but did not inhibit the Li-Na countertransport (Table X). This may indicate that Na-K cotransport is a neutral carrier loaded with Na:K:Cl as described by Geck and Heinz (1980) in Erlich ascites tumor cells. These results are in agreement with those of Chipperfield (1980) and Dunham and Sellers (1980). Dunham et al. (1980) described in human red cells a ouabain-insensitive, furosemide-sensitive Na influx that was significantly lower than the furosemide-sensitive, chloride-dependent K efflux and influx, which suggests the simultaneous presence of a self-exchange pathway for K (Bakker-Grunwald, 1978; Geck and Heinz, 1980).

The data presented in this paper indicate the occupancy by lithium of Na transporting sites in the counter- and cotransport systems. The furosemide-sensitive, chloride-dependent Na-K or Li-K cotransport system uses the energy of the Na and K gradients to drive the coupled movement of a cation partner present in the cis side of the membrane. The phloretin-sensitive Na countertransport system uses the Na or Li gradient, but not the K gradient, to exchange Na for Na or Na for Li in a 1:1 fashion.
Although these two types of Na transport systems of human red cells are phenomenologically different, we cannot, at present, rule out that they are both carried out by the same membrane molecules. The Na-Na exchange system may represent a smaller unit of a larger molecular complex for Na transport. The cotransport unit might be a more complex molecule(s) that can couple the movement of a co-ion against an electrochemical gradient. It is even possible that the Na-Na exchange system might reflect processes of synthesis, activation or degradation or more complex Na transport units such as the Na pump, cotransport, or the Na/Ca exchange system.

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