Lithium Transport Pathways in Human Red Blood Cells

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ABSTRACT   In human red cells, Li is extruded against its own concentration gradient if the external medium contains Na as a dominant cation. This uphill net Li extrusion occurs in the presence of external Na but not K, Rb, Cs, choline, Mg, or Ca, is ouabain-insensitive, inhibited by phloretin, and does not require the presence of cellular ATP. Li influx into human red cells has a ouabain-sensitive and a ouabain-insensitive but phloretin-sensitive component. Ouabain-sensitive Li influx is competitively inhibited by external K and Na and probably involves the site on which the Na-K pump normally transports K into red cells. Ouabain does not inhibit Li efflux from red cells containing Li concentrations below 10 mM in the presence of high internal Na or K, whereas a ouabain-sensitive Li efflux can be measured in cells loaded to contain 140 mM Li in the presence of little or no internal Na or K. Ouabain-insensitive Li efflux is stimulated by external Na and not by K, Rb, Cs, choline, Mg, or Ca ions. Na-dependent Li efflux does not require the presence of cellular ATP and is inhibited by phloretin, furosemide, quinine, and quinidine. Experiments carried out in cells loaded in the presence of nystatin to contain either only K or only Na show that the ouabain-insensitive, phloretin-inhibited Li movements into or out of human red cells are stimulated by Na on the trans side and inhibited by Na on the cis side of the red cell membrane. The characteristics of the Na-dependent unidirectional Li fluxes and uphill Li extrusion are similar, suggesting that they are mediated by the same Na-Li countertransport system.

INTRODUCTION

Li salts are effective in the treatment of mania and are useful in reducing the relapse and recurrence of manic-depressive episodes. In clinical studies, it has been found that the concentration of Li in red cell water during Li administration is usually only about one-third of that in blood plasma (Mendels and Frazier, 1973). Inasmuch as the steady-state concentration ratio between red cells and plasma for a passively distributed cation would be ~1.2, this observa-

tion suggests the presence of an uphill extrusion mechanism for Li. Recently Haas et al. (1975) reported the presence of a ouabain-insensitive Na-Li counter-transport, producing uphill Li extrusion in normal human red cells. This observation has independently been confirmed by Duhm et al. (1976). In the present paper we report experiments which delineate the basic characteristics of different pathways for Li transport. Some of these results have already been reported in preliminary communications (Pandey et al., 1976; Sarkadi et al., 1977).

MATERIALS AND METHODS

Chemicals

KCl, NaCl, MgCl$_2$, glycylglycine, sucrose (Mallinckrodt, Inc., St. Louis, Mo.), Tris-OH (Fisher Scientific Co., Pittsburgh, Pa.); and choline chloride (Eastman Organic Chemicals Div., Eastman Kodak Co., Rochester, N. Y.) were of reagent grade. Ouabain (Sigma Chemical Co., St. Louis, Mo.) was dissolved in isosmotic Tris-HCl, pH 7.4; phloretin (ICN, Nutritional Biochemicals Div., Cleveland, Ohio), furosemide (Fisher Scientific Co.), chlorobutanol (Richter), oligomycin, ethacrynic acid, amiloride (Merck Chemical Div., Merck & Co., Rahway, N. J.), quinine, quinidine, and quinaldine were dissolved in dimethylsulfoxide. Dimethylsulfoxide concentration in the final incubation media was always <1%.

All the solutions for the incubation media had osmotic concentrations of 296–300 mosM. Isosmotic washing solutions were buffered to pH 7.4 by 10 mM Tris-HCl.

Preparation of Red Cells

Human red blood cells were drawn from healthy donors into heparin. The cells were spun down at 10,000 rpm at 4°C in a Sorvall RC 5 (DuPont Instruments-Sorvall, Wilmington, Del.) centrifuge for 10 min. The plasma and the buffy coat were removed by suction, and the cells were then washed three times with isosmotic KCl or MgCl$_2$, depending upon the type of experiment.

To determine dry weight, cellular hemoglobin, and ion concentrations, aliquots of washed red cells were pipetted into nylon tubes (70 mm long, ID 3 mm) and centrifuged at 20,000 g for 15 min at 4°C. After centrifugation the packed cells were isolated by cutting the tube 1 mm below the cell-medium interface. Hemoglobin was measured in the packed wet cells by the cyanomethemoglobin method. For measuring wet weight/dry weight ratio, the cells were weighed, dried overnight, cooled in a desiccator, and weighed again.

Loading of Red Cells with Li

Washed red cells were suspended (hematocrit 20%) either in a 40 mM LiCl (plus 95 mM NaCl, 5 mM KCl, 20 mM Tris-HCl, or glycylglycine, pH 7.4) or in a 140 mM LiCl (plus 20 mM Tris-HCl) medium and incubated for 1 h in a shaker water bath at 37°C. In the former case, the final intracellular Li concentration was 0.7–0.9 mM, and in the latter case 2.8–3.5 mM.

Loading of Red Cells by the Nystatin-Technique

We prepared red cells with either a single cation species or a given proportion of Na, K, or Li using the technique of Cass and Dalmark (1973). Briefly this included washing and suspending cells to a 1% hematocrit in 150 mM cation-chloride, 27 mM sucrose, pH 7.2 at

4°C for 20 min with 0.1 mg/ml nystatin. After a second incubation at 4°C for 20 min, but now without added nystatin, the cells were washed twice with nystatin-free medium containing 1% albumin, and twice with isosmotic $MgCl_2$ at 25°C. As did Cass and Dalmark (1973), we found this procedure adequate to restore normal cation permeability.

Li Efflux Measurements

Li-loaded red cells were suspended to a hematocrit of 5% in the desired medium at pH 7.4, 37°C. At timed intervals, aliquots of the suspension were removed and centrifuged for 1 min at 10,000 g at 4°C. The supernate was removed for cation analysis. At the conclusion of the experiment, aliquots of the suspension were removed and the cells were hemolyzed with distilled water. This sample was then analyzed for total cation concentration and hemoglobin concentration. All incubations and samplings were performed in duplicate.

Li Influx Measurements

Fresh washed red cells were incubated at 37°C in media containing various concentrations of Li. Samples were removed at appropriate intervals and centrifuged. Cells were prepared for determination of Li concentration by washing three times in isosmotic $MgCl_2$ solution, and lysing in distilled water.

ATP Depletion

Red cells were ATP-depleted by incubation for 2 h at 37°C, pH 7.4, in the presence of 10 mM inosine and 2 mM iodoacetate as described by Szasz et al. (1977). Cellular ATP after this depletion is below 10 mmol/liter of cells. Similar results were obtained when ATP was depleted by incubating cells without substrate for as long as 36 h.

Analytical Procedures

Li, K, and Na in cell lysates and in supernates were analyzed by atomic absorption spectrophotometer using a Perkin-Elmer (model 460, Perkin-Elmer Corp., Instrument Div., Norwalk, Conn.) absorption spectrophotometer. Hemoglobin was determined by dilution of the cell lysate with cyanomethemoglobin reagent and measuring the optical density at 540 nm using a Gilford (model 260, Gilford Instrument Laboratories Inc.) spectrophotometer (Van Kampden and Zijlstra, 1961).

Calculations

In the flux experiments, all the changes in cellular ion concentrations were related to the hemoglobin concentration measured in the cell lysates in which ion concentrations were also measured. By using the values obtained by the nylon tube technique for the original cellular hemoglobin and water content, the data were converted to the units of millimoles/liter of original cells or millimoles/liter of original cell water. Influxes and effluxes of Li were calculated from the slopes of the relation between cell Li concentration in millimoles/liter of original cells and time in hours. Usually four points were taken and the slope was determined by linear regression. Only the initial linear parts of these concentration-time curves were used to compute fluxes.

RESULTS

Uphill Li Extrusion from Red Cells

Freshly drawn red cells were loaded with Li to obtain intracellular Li concentrations between 0.7 and 1.0 mmol/liter cells and then incubated in media with

different cations but always including 1.5 mM Li. Cellular Li concentration was determined at the beginning of the experiment and after a 4-h incubation at 37°C, pH 7.4 (Table I). In cells incubated in high Na medium, the cellular Li concentration significantly decreased in spite of the inward Li concentration

TABLE I

EFFECTS OF EXTERNAL CATIONS ON UPHILL Li EXTRUSION
FROM RED CELLS

| | Li concentration | | | |
| | | Red cells | | |
External salt	Medium	0 h	4 h	Change/4 h
mM	mM		mmol/(liter of cells)	
NaCl (140)	1.50	0.70	0.30	−0.40
KCl (140)	"	"	0.81	+0.11
CsCl (140)	"	"	0.77	+0.07
RbCl (140)	"	"	0.90	+0.20
Choline Cl (140)	"	"	0.81	+0.11
MgCl₂ (110)	"	"	0.80	+0.10
CaCl₂ (110)	"	"	0.70	0.00

Cells were preloaded with Li by a 1-h incubation in a medium containing 40 mM LiCl, 95 mM NaCl, 5 mM KCl, and 20 mM glycylglycine, pH 7.4, 37°C. The Li-preloaded cells were incubated in the media indicated plus 1.5 mM LiCl and 20 mM glycylglycine, pH 7.4, at 37°C. Hematocrit was 5%. Intracellular Li concentration was determined after washing the cells three times in cold MgCl₂ solution.

TABLE II

EFFECT OF OUABAIN AND PHLORETIN ON UPHILL Li EXTRUSION FROM
RED CELLS

| | | | | Li concentration | | |
| | Addition | | | Red cells | | |
External salt	Ouabain	Phloretin	Medium	0 h	4 h	Change/4 h
mM			mM		mmol/(liter of cells)	
NaCl (140)	0	0	1.50	1.04	0.60	−0.44
"	+	0	"	"	0.50	−0.54
"	0	+	"	"	1.08	+0.04
"	+	+	"	"	0.94	−0.10
MgCl₂ (110)	0	0	1.50	1.04	1.80	+0.76
"	+	0	"	"	1.30	+0.26
"	0	+	"	"	1.87	+0.83
"	+	+	"	"	1.26	+0.22

For the conditions of Li loading and incubation of Li-loaded cells see the legend of Table I. The concentrations of ouabain and phloretin were 10^{-4} M.

gradient. In cells incubated in different Na-free media, the cellular Li concentration remained unchanged (CaCl₂) or increased.

Table II shows the effect of ouabain and phloretin on the changes in cellular Li concentration during a 4-h incubation in a NaCl or in a MgCl₂ medium. The

decrease in cellular Li concentration in the NaCl medium (uphill net extrusion) was slightly enhanced by ouabain and completely inhibited by phloretin. Addition of 1 mM furosemide, quinine, or quinidine resulted in similar inhibition of the net Li extrusion in a NaCl medium. In the cells incubated in $MgCl_2$ medium, intracellular Li concentration increased above the medium Li concentration during 4 h. This increase was inhibited by ouabain in the presence or absence of phloretin.

Fig. 1 shows the changes in intracellular Li concentration in a 3-h incubation
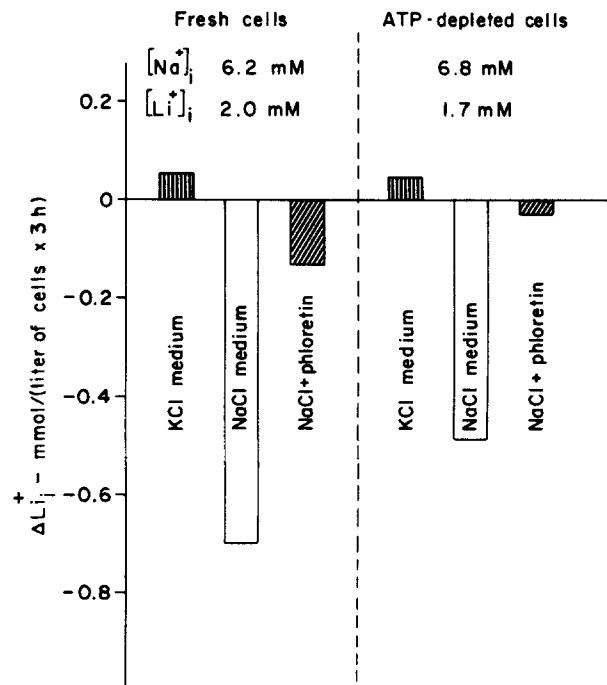

FIGURE 1.   Effect of ATP depletion on the net Li extrusion from red cells. Red cells were loaded with Li by incubation for 2 h at 37°C in a medium containing 40 mM LiCl, 95 mM KCl, 10 mM inosine, and 20 mM Tris-HCl, pH 7.4. During Li loading, a portion of red cells was depleted of ATP by addition of 2 mM iodoacetate and 10 mM inosine to the incubation medium. Li-loaded cells with ATP contained 2.0 mmol Li/liter cells, while in ATP-depleted cells the Li concentration was 1.7 mmol/liter cells. The cells were incubated then in either 140 mM NaCl or 140 mM KCl media, supplemented with 3 mM LiCl, 1 mM ouabain, and 20 mM Tris-HCl, pH 7.4, at 37°C. Hematocrit was 5%. The phloretin concentration was 2×10^{-4} M.

period in Li-loaded fresh and ATP depleted red cells. The cells were ATP depleted during the Li loading as described in the Methods section. Clearly, Na-stimulated, phloretin-sensitive uphill Li extrusion was present in cells with an ATP concentration <10 μmol/liter of cells.

To investigate the unidirectional components of these net changes in cellular

Li concentration, we studied Li influx and efflux in normal cells and in cells with altered cation composition.

Li Influx into Normal Red Cells

Li influx was studied by incubating washed red cells in media with different cation compositions, supplemented with 10 mM LiCl (Table III). Li influx was the greatest in choline Cl and in $MgCl_2$ media, lower in NaCl, and the lowest in a KCl medium. Addition of ouabain greatly reduced Li influx in $MgCl_2$ or in choline Cl, slightly in NaCl, and caused no change in a KCl medium. The ouabain-sensitive component of Li influx thus was small in the presence of

TABLE III

Li INFLUX INTO NORMAL RED CELLS: EFFECT OF OUABAIN, PHLORETIN, AND EXTERNAL CATIONS

	Addition		Li influx		
External salt	Ouabain	Phloretin	Control	Ouabain-sensitive	Phloretin-sensitive
mM			*mmol/(liter of cells × h)*		
NaCl (130)	0	0	0.42		
"	+	0	0.29	0.13	
"	+	+	0.18		0.11
KCl (130)	0	0	0.33		
"	+	0	0.33	0.00	
"	+	+	0.18		0.15
Choline Cl (130)	0	0	0.67		
"	+	0	0.27	0.40	
"	+	+	0.13		0.14
$MgCl_2$ (110)	0	0	0.68		
"	+	0	0.30	0.38	
"	+	+	0.15		0.15

Washed red cells were incubated in the media indicated plus 10 mM LiCl and 20 mM glycylglycine, pH 7.4, at 37°C. Hematocrit was 5%. The concentrations of ouabain and phloretin were 10^{-4} M. Samples were taken in 30-min periods for 2 h, and intracellular Li concentration was determined after washing the cells in a cold $MgCl_2$ solution.

external Na and absent in the presence of external K. In the presence of ouabain, addition of phloretin resulted in a reduction of Li influx in each medium. The ouabain-insensitive, phloretin-inhibited component was less in a NaCl medium compared with KCl, $MgCl_2$, and choline-Cl media, in which it had similar values.

The dependence of Li influx on external Li concentration was measured in a $MgCl_2$ medium in which the ouabain-sensitive and the phloretin-sensitive components could be studied simultaneously (Fig. 2). Ouabain inhibited a saturable component of Li influx which had a maximum rate of ~ 0.5 mmol/ (liter of cells × hour) and was saturated by about 15 mM external Li (Li_o). The

ouabain-insensitive Li influx at low external Li concentrations (below 20 mM) was a nonlinear function of Li_o, whereas, in the presence of ouabain plus phloretin, Li influx was decreased and a linear function of Li_o over the entire concentration range investigated. (A detailed kinetic analysis of phloretin-sensitive Li influx and Li efflux is presented in the accompanying paper.)

To characterize further the ouabain-sensitive component, Li influx was measured at two different external Li concentrations (3.2 and 32 mM) as a function of extracellular K when substituted for external choline (Fig. 3). External K at low concentrations inhibited the total and the ouabain-sensitive Li
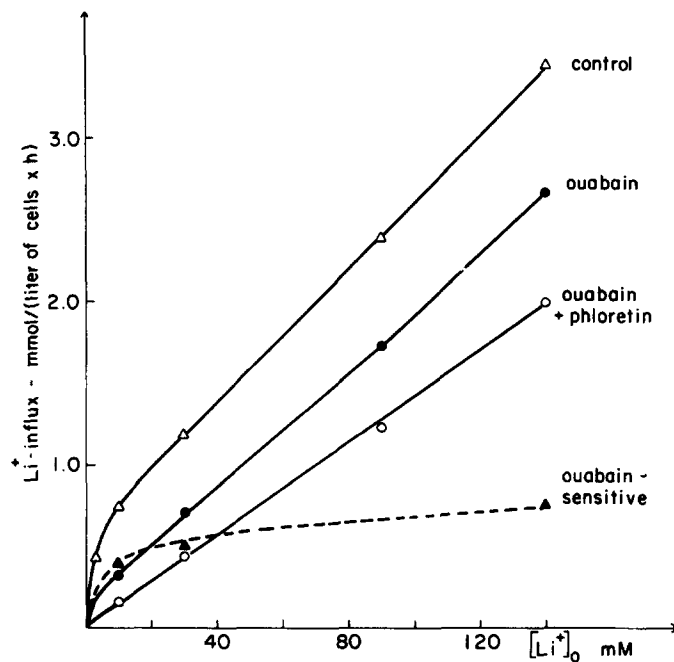

FIGURE 2. Li influx into red cells as a function of external Li concentration. Washed red cells were incubated in media containing increasing concentrations of LiCl substituted for $MgCl_2$. The media contained also 20 mM glycylglycine, pH 7.4. Hematocrit was 5%, incubation temperature 37°C. Ouabain and phloretin concentrations were 10^{-4} M.

influx at both Li concentrations but more effectively at the lower Li_o. At 3.2 mM Li_o, half maximum inhibition was obtained at an external concentration of < 2 mM.

Li Efflux from Normal Red Cells

Li efflux was measured from Li-loaded cells into media with different cation composition with and without ouabain and phloretin (Fig. 4). In cells containing 2.9 mmol Li/liter cells, Li efflux into choline-Cl, KCl, or $MgCl_2$ media was ~ 0.2 mmol/(liter of cells × hour) and was about doubled in a NaCl medium. Phloretin

did not affect Li efflux into Na-free media, whereas in NaCl medium Li efflux was progressively inhibited by increasing concentrations of phloretin. Li efflux in NaCl medium was maximally inhibited by 2.5×10^{-4} M phloretin (hematocrit 5%), and the residual Li efflux was the same as in media without Na.

Fig. 5 shows the effect of four drugs on the Na-stimulated Li efflux from red cells. 2.5×10^{-4} M phloretin and 10^{-3} M furosemide decreased Li efflux in a
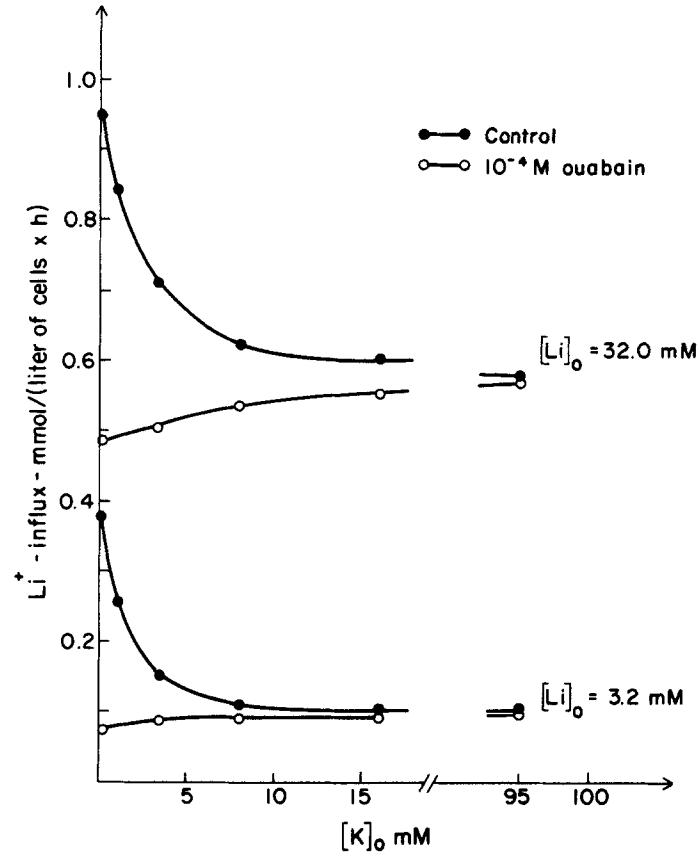

FIGURE 3.   Effect of external K on Li influx into red cells. Washed red cells were incubated in media with increasing KCl concentrations substituted for choline Cl. The media contained either 32 or 3.2 mM LiCl and also 20 mM Tris-HCl, pH 7.4. Hematocrit was 5%, incubation temperature 39°C. (●) Control; (○) 10^{-4} M ouabain.

NaCl medium to the rate observed in a KCl medium but had no effect on Li efflux in a KCl medium. Ethacrynic acid (10^{-3} M) caused about 50% inhibition of the Na-dependent Li efflux. Higher doses of ethacrynic acid resulted in increasing hemolysis and thus increased Li efflux. From among drugs tested, quinine and quinidine in 10^{-3} M concentrations proved as powerful inhibitors of Na-dependent Li efflux as phloretin. No appreciable effect on Li efflux was

observed in the presence of 1–3 mM of chlorobutanol (which is a powerful inhibitor of the Ca dependent K transport in red cells), 10^{-5} to 10^{-7} M amiloride, 10^{-4} M dipyridamole, 10 μg/ml oligomycin, 10^{-3} M quinaldine, 10^{-3} M harmine, and 10^{-3} M dilantin.

To examine the effect of ATP depletion on the Na-dependent Li efflux we treated red cells with iodoacetate and inosine and then loaded with Li as before.
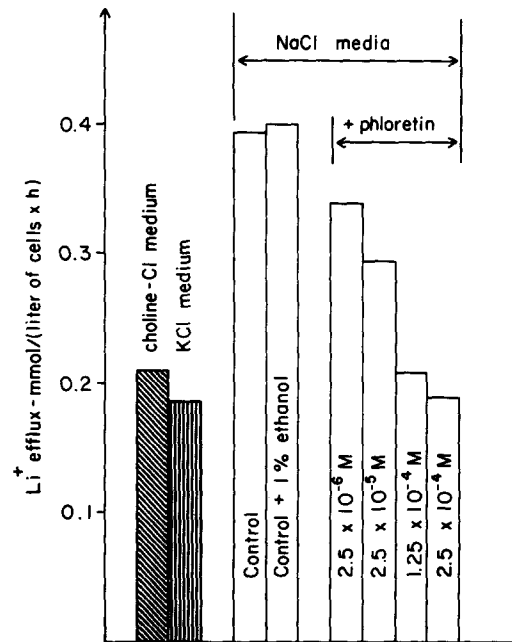

FIGURE 4. Li efflux into different incubation media—effects of ouabain and phloretin. Red cells were preloaded with Li by a 1-h incubation at 37°C in a medium containing 140 mM LiCl and 20 mM Tris-HCl, pH 7.4. The intracellular Li concentration obtained was 2.9 mmol/liter cells. Li efflux was measured at 37°C into media containing either 140 mM choline Cl, 140 mM KCl, 140 mM NaCl, or 80 mM MgCl₂ + 70 mM sucrose. The media were supplemented with 20 mM Tris-HCl, pH 7.4.

ATP depletion did not affect Li efflux either into a Na-free medium or into solutions containing Na as the dominant cation.

Li Influx and Efflux in Red Cells with Altered Na and K Concentrations

Cells were prepared by the nystatin method (see Methods) to contain either NaCl (<1 mmol K/liter cells) or KCl (<0.1 mmol Na/liter cells). Li influx was measured into both Na and K cells suspended in either KCl or NaCl media

supplemented with 10 mM LiCl. Li efflux was measured from Na cells and K cells containing 10 mmol Li/liter cells.

Li influx was significantly higher into Na cells than into K cells in both KCl and NaCl media (Table IV). As shown in Table V, ouabain-sensitive Li influx was greatest into Na cells incubated in NaCl medium and about half as much into K cells incubated in NaCl medium. No ouabain-sensitive Li uptake was observed in the presence of high external K concentrations. Phloretin-sensitive Li influx was about twice as great into Na loaded cells when the external cation was K rather than Na. No phloretin-sensitive Li uptake was observed in K cells incubated in either medium. The residual Li influx in the presence of 10^{-4} M
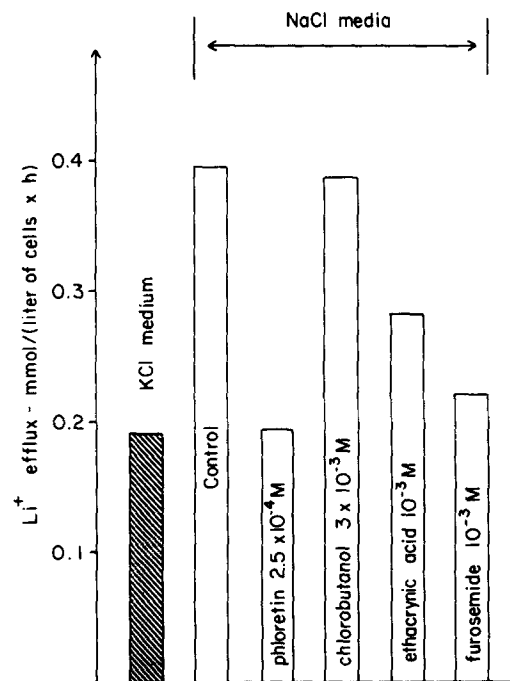

FIGURE 5. Effect of drugs on Li efflux from red cells. For the conditions of Li loading and Li efflux measurement see the legend of Fig. 4. Intracellular Li concentration was 2.6 mmol/liter cells.

ouabain and 10^{-4} M phloretin was greater into Na cells, most probably because of an incomplete inhibition of the Na-dependent Li movement by this concentration of phloretin.

Li efflux was large when either Na or K loaded red cells were placed into a NaCl medium, and was small when the external cation was K (Table IV). As shown in Table V, no ouabain-sensitive Li efflux was observed from either Na or K cells when internal Li concentration was 10 mmol/liter cells. Phloretin-sensitive Li efflux was negligible if the external medium contained only K. Residual Li efflux was almost twice as great into a NaCl rather than a KCl medium, again probably as a result of incomplete inhibition by phloretin.

According to these experiments, ouabain-sensitive Li influx is stimulated by

internal Na and blocked by external K. Phloretin-sensitive Li transport requires the presence of Na on the *trans* side and is inhibited by Na on the *cis* side of the membrane.

To test for the presence of a ouabain-sensitive Li efflux from cells containing neither Na nor K, we prepared cells by nystatin method which contained only 150 mmol Li/liter cells (K concentration below 1 mmol/liter cells, Na concentration below 0.1 mmol/liter cells). A ouabain-sensitive Li efflux of 0.58 mmol/(liter of cells × hour) into a NaCl medium, and 1.1 mmol/(liter of cells × hour) into a

TABLE IV

EFFECTS OF Na AND K ON Li INFLUX AND Li EFFLUX IN RED CELLS

Cation		Li influx			Li efflux		
External	Internal	Control	+Ouabain	+Ouabain +Phloretin	Control	+Ouabain	+Ouabain +Phloretin
		mmol/(liter of cells × h)					
K	Na	0.78	0.80	0.32	0.13	0.13	0.13
K	K	0.13	0.13	0.13	0.13	0.14	0.12
Na	Na	0.77	0.50	0.27	0.40	0.38	0.20
Na	K	0.23	0.12	0.12	0.50	0.49	0.17

Red cells were loaded with Na or K by the nystatin method. In K cells, Na concentration was <0.1 mmol/liter of cells; in Na cells, K concentration was <1 mmol/liter of cells. For efflux experiments, the cells also contained 10 mmol Li/liter of cells. The nystatin loaded cells were incubated in 130 mM NaCl or 130 mM KCl media supplemented with 10 mM LiCl in the influx experiments, and in 140 mM NaCl or 140 mM KCl media without external Li in the efflux experiments. The media also contained 20 mM glycylglycine buffered to pH 7.4. Hematocrit was 5%. Ouabain and phloretin concentrations were 10^{-4} M.

TABLE V

COMPONENTS OF Li INFLUX AND LI EFFLUX IN RED CELLS

Cation		Li influx			Li efflux		
External	Internal	Ouabain-sensi-tive	Phloretin-sensitive	Residual	Ouabain-sensi-tive	Phloretin-sensitive	Residual
		mmol/(liter of cells × h)					
K	Na	−0.02	0.48	0.32	0.00	0.00	0.13
K	K	0.00	0.00	0.13	−0.01	0.02	0.12
Na	Na	0.27	0.23	0.27	0.02	0.18	0.20
Na	K	0.11	0.00	0.12	0.01	0.32	0.17

For the conditions of loading and incubation of red cells see the legend of Table IV.

KCl medium was measured (Table VI). This observation extends the data of Dunham and Senyk (1977) and is consistent with their proposal that Li can be transported outwards by the Na-K pump in the absence of internal Na and K.

DISCUSSION

The findings in this paper support the concept that the following separable pathways are present for Li transport in human red cells: (*a*) ouabain-sensitive Li transport; (*b*) the Na-dependent, ouabain-insensitive, phloretin-sensitive

pathway which is responsible for Li-Na counterflow; and (c) a residual pathway that is insensitive to drugs (Fig. 6). Experiments reported elsewhere indicate the presence of at least three additional pathways. (d) In bicarbonate-containing media, $LiCO_3$ ion pair formation results in Li transport on the system which facilitates monovalent anion exchange (Funder and Wieth, 1967; Duhm et al., 1977; Funder et al., 1978). (e) A system with a low affinity for Li may accomplish Li influx in exchange for K and Na efflux from cells suspended in media containing Li as the only monovalent cation (Sarkadi et al., 1978). (f) The choline exchange system described by Martin (1972) has a low affinity for Li.

The ouabain-sensitive Li transport in human red cells is probably mediated

TABLE VI

EFFECT OF OUABAIN ON Li EFFLUX FROM Li-LOADED
(Na- AND K-FREE) CELLS

Medium	Li efflux		
	Control	Ouabain	Ouabain-sensitive
	mmol/(liter of cells × h)		
NaCl (140 mM)	3.05	2.47	0.58
KCl (140 mM)	3.46	2.36	1.10

Red cells were loaded with Li by the nystatin method. Intracellular Na concentration was <0.1 mmol/liter of cells, K concentration <1 mmol/liter of cells. Cells were incubated in either a 140 mM NaCl or in a 140 mM KCl medium supplemented with 20 mM glycylglycine, pH 7.4. Hematocrit was 5%, incubation temperature 37°C. The concentration of ouabain was 10^{-4} M.
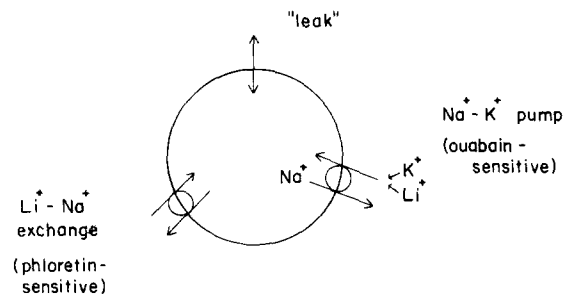

FIGURE 6.   Li transport pathways in human red cells.

by the Na-K pump. If the external solution does not contain Na and K, Li influx into normal red cells through this mechanism has a maximum value of ~ 0.5 mmol/(liter of cells × hour) and is half-maximally stimulated by ~15 mM external Li. External K inhibits ouabain-sensitive Li uptake in a competitive manner, and half-maximal inhibition of ouabain-sensitive Li influx is caused by < 2 mM K when external Li concentration is 3.2 mM (Fig. 3). External Na is also an inhibitor of ouabain-sensitive Li influx though less so than K (Table III). In the presence of intracellular Na and (or) K, no ouabain-sensitive Li efflux was observed. However, when red cells were loaded with 150 mM LiCl with no intracellular K or Na present, a ouabain-sensitive Li efflux could be measured,

which was stimulated by external K. In Na-K-free cells, a similar ouabain-sensitive Li efflux was detected by Dunham and Senyk (1977). Thus, the Na-K pump can transport Li in either direction. Reaction of the Na-K pump and Na-K ATPase with Li has been previously reported (Skou, 1965; Maizels, 1968; Sachs and Welt, 1967). Comparing these data with our present studies we conclude that the affinity of the external (K) site of the Na-K pump for Li is lower than for K but higher than for Na. The affinity of the internal (Na) site for Li is much lower than for Na, because, at about equal internal Li and Na concentrations, there is no detectable Li efflux through this mechanism. The maximum rate of ouabain-sensitive Li influx or Li efflux is about one-fifth that for K or Na. Under in vivo conditions, at normal K and Na plasma concentrations and at Li concentrations used therapeutically (0.5–1.2 mM) Li movement through the Na-K pump, according to these figures, is negligible.

The ouabain-insensitive Li transport in human red cells has a Na-dependent component which can accomplish uphill Li movement (Haas et al., 1975; Duhm et al., 1976; Duhm and Becker, 1977 *a, b, c* Table I). Na-dependent uphill Li extrusion is inhibited by phloretin, furosemide, quinine, and quinidine and the operation of this pathway does not require the presence of cellular ATP (Table II, Fig. 1). The unidirectional, ouabain-insensitive Li movements are stimulated by Na on the *trans* side and are inhibited by Na on the *cis* side of the membrane. No other cation tested can substitute for Na in this role. Na-dependent Li fluxes are inhibited by phloretin, furosemide, quinine, and quinidine and do not require the presence of ATP. The similar characteristics of the uphill Li extrusion and the Na-dependent unidirectional Li fluxes suggest that they are mediated by the same process, that is, by Na-Li countertransport. The kinetics and stoichiometry of this pathway are the subject of the accompanying paper.

The residual pathway which is insensitive to ouabain, phloretin, furosemide, quinine, and quinidine is a linear function of the Li concentration gradient (Fig. 2). This "leak" transport of Li occurs in the presence of ouabain when Na ions are absent from both sides of the membrane. The same Li leak flux is observed in the simultaneous presence of Na ions and phloretin, and phloretin has no effect on Li movement in the absence of Na. In red cells loaded in the presence of nystatin, the residual Li fluxes were observed to be higher in the presence of Na on the *trans* side of the membrane (Table IV). This phenomenon is most probably due to an incomplete inhibition of the Na-dependent Li transport by phloretin. The extent of the inhibition by phloretin is determined by the ratio of the number of red cells to the number of phloretin molecules, and this ratio may be altered by various pretreatments of red cells.

Inasmuch as all the experiments reported in this paper were carried out in the absence of bicarbonate in the incubation media, the bicarbonate-dependent Li transport (Funder and Wieth, 1967; Duhm and Becker, 1977 *a*; Funder et al., 1978) is outside of our scope. Extrapolating their data, the contribution of the bicarbonate-stimulated Li fluxes in our experiment was negligible at the low bicarbonate concentrations present in the solutions equilibrated with air. In the following paper we also present data for this Li transport pathway.

In conclusion, we emphasize that the only transport system that can produce net uphill extrusion of Li from human red cells under in vivo conditions, and

thus that can produce lower Li concentrations in red cells than in the plasma, is Li-Na counterflow on the Li-Na exchange system.

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