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Studies on Lithium Transport Across the Red Cell Membrane

V. On the Nature of the Na^+-Dependent Li^+ Countertransport System of Mammalian Erythrocytes

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Summary. Ouabain-resistant Na^+-Li^+ countertransport was studied on erythrocytes of man, sheep, rabbit, and beef. A transport system, exchanging Li^+ for Na^+ in a ratio of 1:1, was present in all four species. Li^+ uptake by the exchange system increased 30-fold in the order man < HK-sheep < LK-sheep < rabbit < LK-beef. This order is identical to that of ouabain-resistant Na^+-Na^+ exchange in these species, but bears no relation to the Na^+-K^+ pump activity. The activity of the Na^+-Li^+ exchange system varied up to 7 and 16-fold among individual red cell specimens from man and beef, the variability being much smaller in sheep and rabbit erythrocytes. The affinities of the system for Li^+ and Na^+ were similar among the species and individuals (half saturation of the external site at about 1 mM Li^+ and 50 mM Na^+, respectively).

50–60% of Na^+-Li^+ exchange was blocked by N-ethylmaleimide in all species. *p*-Chloromercuribenzene sulfonate inhibited the exchange only in beef and sheep erythrocytes (60–80%). The two SH-reagents act by decreasing the maximum activity of the system, whilst leaving its affinity for Li^+ unaltered. Phloretin was a potent inhibitor in all species. 1 mM each of furosemide, ethacrynic acid, and quinidine induced only a slight inhibition. The Na^+-Li^+ exchange of human and beef erythrocytes increased 3.5-fold upon elevation of the extracellular pH from 6 to 8.5, the pH-dependence arising from a change in affinity of the system for the cations and being similar to that reported for ouabain-resistant Na^+-Na^+ exchange in beef erythrocytes.

It is concluded that a transport system exists in the red cell membranes of the four species which can mediate ouabain-resistant exchange of either Na^+ for Na^+, Na^+ for Li^+, or Li^+ for Li^+. The exchange system exhibits essentially identical transport characteristics in the four species, but shows a marked inter- and intra-species variability in maximum transport capacity and some differences in susceptibility towards inhibitors. A similar transport system is probably present also in other tissues. The exchange system seems to be distinct from the conventional Na^+-K^+ pump and shows no clear relation to one of the furosemide-sensitive, ouabain-resistant Na^+ transport systems described in the literature.

Recently we have characterized a ouabain-resistant Na^+-Li^+ countertransport system which can mediate Li^+ transport in both directions across the membrane of human erythrocytes and human red cell ghosts

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(Duhm *et al.*, 1976). The same system has coincidently been described by Haas, Schooler and Tosteson (1975). Na^+-Li^+ countertransport is passive in nature and independent of energy supply by ATP. A secondary active Li^+ uphill transport can be driven in either direction, if a driving force is provided by an oppositely directed Na^+ gradient. The countertransport shows saturation kinetics for both internal and external Li^+ and Na^+, the two ions competing with one another when present at the same side of the membrane (Duhm *et al.*, 1976, Duhm & Becker, 1977*a, b*). These findings have been confirmed by Pandey *et al.* (1978) and by Sarkadi *et al.* (1978). Effective inhibitors of the Na^+-Li^+ exchange system are N-ethylmaleimide and phloretin (Duhm & Becker, 1977*b*; Sarkadi *et al.*, 1978; Funder & Wieth, 1978).

Evidence has been obtained that the countertransport system exchanges Li^+ for Na^+ in a ratio of 1:1 in human (Sarkadi *et al.*, 1978) and beef erythrocytes (Funder & Wieth, 1978). Hence, the Na^+-Li^+ countertransport system exhibits all the properties of an exchange system as originally postulated by Ussing (1949).

A peculiarity of the Na^+-Li^+ exchange system of human erythrocytes is the marked interindividual variability in its maximum activity (Duhm & Becker, 1977*b*; Greil *et al.*, 1977; Sarkadi *et al.*, 1978), this apparently being genetically determined (Dorus *et al.*, 1974; Schless *et al.*, 1975; Duhm & Becker, 1977*b*; Mendlewicz *et al.*, 1978). The other pathways of Li^+ transport across the red cell membrane, i.e., the Na^+-K^+ pump (Duhm & Becker, 1977*a*; Dunham & Senyk, 1977; Sarkadi *et al.*, 1978), the anion exchange system (Duhm & Becker, 1977*a*, 1978*a*; Becker & Duhm, 1978; Funder, Tosteson & Wieth, 1978; Sarkadi *et al.*, 1978), and residual leak (Duhm & Becker, 1977*b*, 1978*a*; Sarkadi *et al.*, 1978) show no such interindividual variability.

The present paper deals with the relation of the Na^+-Li^+ exchange system to other cation transport systems of mammalian erythrocytes, such as the Na^+-K^+ pump, the furosemide-sensitive "second pump" and the ouabain-resistant Na^+-Na^+ exchange system. In addition to human erythrocytes, red cells of beef, sheep and rabbits are studied because of their characteristic differences in Na^+-K^+ pump and ouabain-resistant Na^+-Na^+ exchange activities. Beef and sheep erythrocytes, for instance, are known to exhibit a much more active ouabain-resistant Na^+-Na^+ exchange, but a lower Na^+-K^+ pump activity than do human erythrocytes (Tosteson & Hoffman, 1960; Sorenson, Kirschner & Barker, 1962), the characteristics of Na^+-Na^+ exchange in beef erythrocytes and its inhibition by SH-reagents having been studied in detail by Motais (1973)

and by Motais and Sola (1973). Furthermore, sheep (and beef) exhibit a genetic dimorphism with respect to their red cell Na^+ and K^+ content and Na^+-K^+ pump activity, respectively (Tosteson & Hoffman, 1960; Motais, 1973). Rabbit erythrocytes were selected as an example of red cells with a higher Na^+-K^+ pump activity and a faster ouabain-resistant Na^+-Na^+ exchange than are found in human erythrocytes (Pfleger, Rummel & Seifen, 1967; Rettori *et al.*, 1969; Villamil & Kleeman, 1969).

Materials and Methods

The studies were performed on fresh blood drawn from the antecubital vein of apparently healthy human males, and from the ear vein of rabbits, heparin being used as anticoagulant. Beef and sheep blood was obtained from the local slaughter house (anticoagulant citrate). Phloretin was from K & K Laboratories, Plainview, N-ethylmaleimide (NEM), *p*-chloromercuribenzene sulfonic acid (PCMBS), and quinidine hydrogen chloride from Sigma, ethacrynic acid from Sharp & Dome, München, furosemide (free acid or K^+ salt) from Hoechst AG, Frankfurt, dipyridamole from Thomae AG, Biberach, and ouabain from Merck AG, Darmstadt.

General Procedure

Erythrocytes were washed three times in the prospective, isotonic incubation medium and resuspended at 37 °C and a hematocrit of 3%. 0.1 mM ouabain was present in all incubation media. The pH was measured regularly during the incubation periods, and adjusted, if necessary. For determinations of *Li^+ uptake* at extracellular Li^+ concentrations up to 10 mM, the cells were preincubated for 20–30 min at the desired pH value (generally pH 7.4) before the Li^+ influx was initiated by adding aliquots of isotonic LiCl stock solution. If Li^+ concentrations above 10 mM were to be tested, erythrocytes were added to prewarmed solutions containing Li^+. *Li^+ release* was studied on cells preloaded with Li^+ in isotonic LiCl to contain 2 μmol Li^+/ml cells, the duration of preloading being empirically determined. After Li^+ loading, the cells were washed in the cold, and suspended in warm, Li^+-free medium. A sample of the suspension was collected immediately to determine the initial red cell Li^+ content.

To alter the Na^+ concentration in human erythrocytes, the cells were incubated for 15 min to 4 hr in K^+ or Na^+ chloride media in which 40 mM Cl^- were replaced by 20 mM sulfite (5 mM glucose, 1 mM inorganic phosphate, pH 7.4, 37 °C, hematocrit 3–5%). Sulfite served to accelerate downhill Na^+ net-transport through the anion exchange system (Becker & Duhm, 1978). The Na^+ media contained 0.1 mM ouabain. Sulfite was subsequently removed by two incubations of 10-min duration in media free of sulfite and four washings at 37 °C.

In experiments performed to study the action of inhibitors, these were added to the media either simultaneously with the red cells, or during the preincubation period 15 min prior to initiation of Li^+ uptake. Phloretin was added as ethanol solution; furosemide and quinidine were solubilized with dimethyl sulfoxide. Stock solutions of ethacrynic acid and dipyridamole were prepared with diluted KOH and HCl, respectively. NEM and PCMBS were dissolved in water. Dimethyl sulfoxide and ethanol did not alter Li^+ transport at the amounts present in the red cells suspensions (0.2%, vol/vol).

After the time intervals indicated in Results, aliquots of the suspensions were rapidly cooled in an ice bath and the cells were washed three times in a tenfold excess of ice-cold, isotonic choline chloride. Finally, the cells were packed by 4 min centrifugation at 10,000 × *g*. Cellular Li^+, Na^+ and K^+ contents and cation concentrations in the media were determined with a Perkin-Elmer atomic absorption spectrophotometer 420 after suitable dilution of the samples in 6% *n*-butanol (vol/vol). The cellular hemoglobin content was determined simultaneously in the red cell sediment by the cyano-methemoglobin method. All cellular Li^+ contents given in Results are corrected to a hemoglobin content (by tetramer) of 5.2 μmol/ml cells.

Results

The time course of ouabain-resistant Li^+ uptake by representative beef, rabbit, and LK-sheep red cell specimens is compared in Fig. 1 with the uptake by erythrocytes of the donor U.L. Pronounced species differences are evident. Li^+ accumulated most rapidly within the beef erythrocytes, an outwardly directed electrochemical Li^+ gradient being established within 1 hr (*see also* Fig. 10). The lowest rate of Li^+ uptake was observed with human erythrocytes, although the cells of the donor studied are characterized by a high activity of the Na^+-Li^+ countertransport system, as compared to other human donors (Duhm & Becker, 1977*b*).

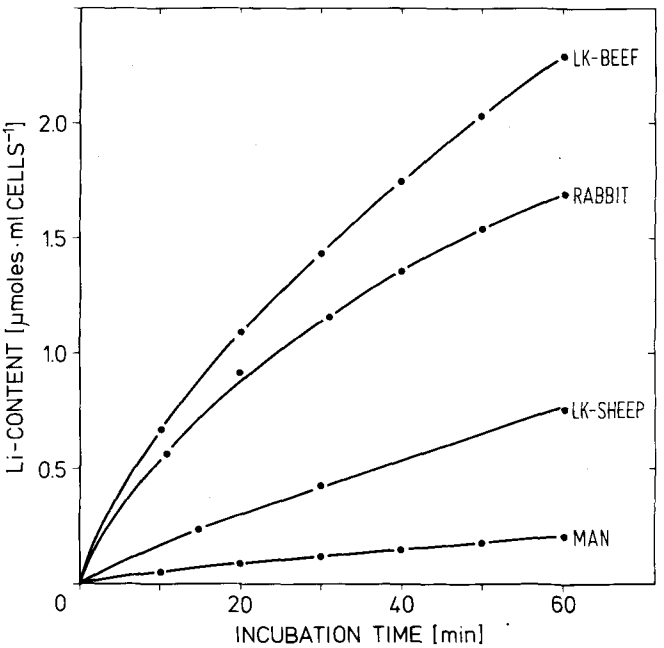

Fig. 1. Species differences in Li^+ uptake. Erythrocytes from an individual LK-beef, rabbit, LK-sheep and from the donor U.L. were incubated in isotonic choline chloride media containing 2 mM Li^+ and 0.1 mM ouabain (pH 7.4, 37 °C, hematocrit 3%)

Na^+ Dependence of Li^+ Transport

Li^+ uptake is inhibited by external Na^+. This is demonstrated in Fig. 2 for the example of rabbit erythrocytes. With 2 mM Li^+ in the medium, half maximum inhibition is attained at about 50 mM external Na^+. Similar results were obtained with human, sheep, and beef erythrocytes. Li^+ uptake was not altered when external choline was replaced by K^+, Mg^{2+} or Ca^{2+} (results not shown).

Conversely, a rise in internal Na^+ concentration accelerates the rate of Li^+ uptake, as shown in Fig. 3*a* for the example of human erythrocytes. The dependence of Li^+ uptake on internal Na^+ was markedly reduced by NEM and fully blocked by phloretin. The NEM- and phloretin-sensitive components of Li^+ uptake are depicted in Fig. 3*b* and yield apparent K_m values for internal Na^+ in the range of 6–8 mM (*see* Legend of Fig. 3). A similar K_m for internal Na^+ (7–8 mM) was derived for rabbit erythrocytes.

Li^+ release also displays a strong dependence on the Na^+ concentration at the trans side of the membrane. Whereas Li^+ release proceeds rather slowly in isotonic choline$^+$, Mg^{2+}, or K^+ chloride media, it is

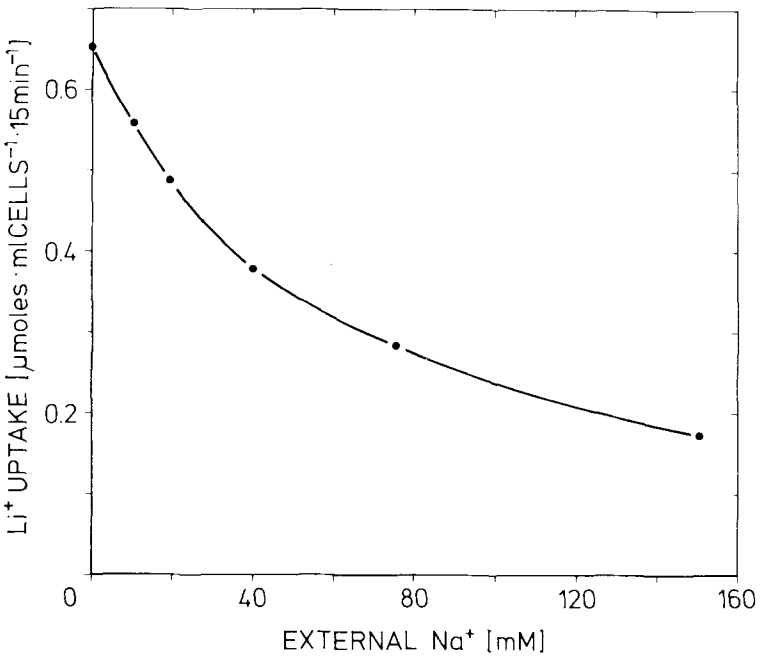

Fig. 2. Inhibition of Li^+ uptake by external Na^+ in rabbit erythrocytes. External choline$^+$ was replaced by Na^+. The uptake period was 15 min. Experimental conditions otherwise were as described in the legend of Fig. 1

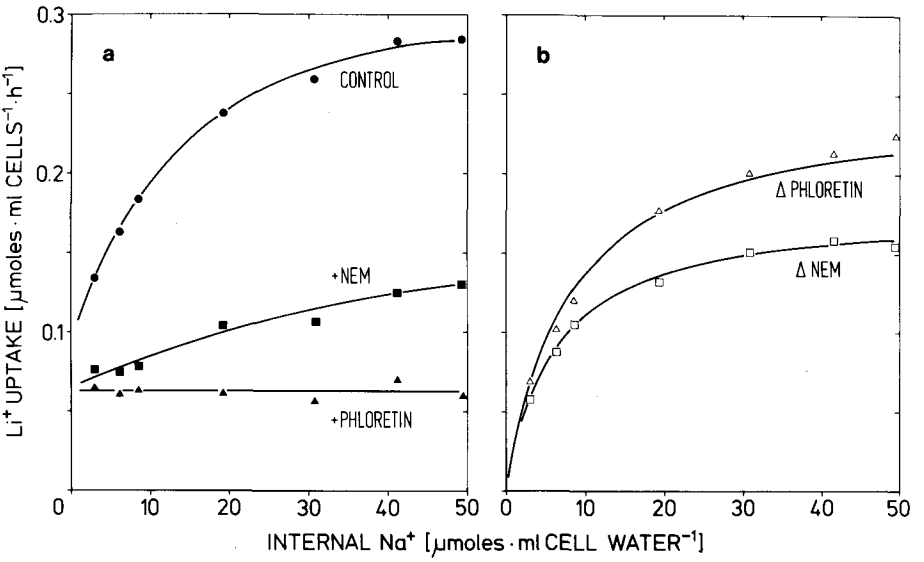

Fig. 3. Dependence of Li^+ uptake on internal Na^+ concentration of human erythrocytes. (*a*): Red cells enriched or depleted of Na^+ (*see* Materials and Methods) were incubated in isotonic choline chloride media containing 2 mM Li^+ and 0.1 mM ouabain, without and with 0.5 mM NEM or 0.2 mM phloretin (pH 7.4, 37 °C, hematocrit 3%, donor J.D.). 4-Acetamido-4′-isothiocyanato stilbene-2,2′-disulfonic acid (SITS, 0.1 mM) was additionally present to preclude an action of residual sulfite and carbonate on Li^+ transport (Becker & Duhm, 1978). (*b*): NEM- and phloretin-sensitive components of Li^+ uptake, estimated from the data given in *a*. The curves represent the equation $V = V_{max}/(1 + K_m/[Na^+_i])$ where V is Li^+ uptake sensitive to the inhibitors, V_{max} is the maximum inhibition of Li^+ uptake induced by NEM and phloretin (0.18 and 0.24 μmol·ml cells^{-1}·hr^{-1}, respectively), K_m is the half saturating concentration of internal Na^+ (6.4 and 7.7 mM for NEM- and phloretin-sensitive uptake, respectively), and $[Na^+_i]$ the internal Na^+ concentration in μmol/ml cell water

accelerated 10 to 50-fold in isotonic NaCl medium in the four species (Fig. 4). The Li^+ release induced by external Na^+ is inhibited by NEM and phloretin in all cases. Furosemide (1 mM) is found to be almost ineffective, as was ethacrynic acid (0.5 and 1 mM, results not shown).

Coupling of Na^+ and Li^+ Movements

The net uptake of Li^+ is associated with a net release of Na^+. In the experiments shown in Fig. 5, rabbit erythrocytes containing 15 μmol Na^+/ml cells (i.e., 23 μmol Na^+/ml cell water) were suspended in a K^+ medium containing 15 mM Na^+ to initially establish a passive Na^+

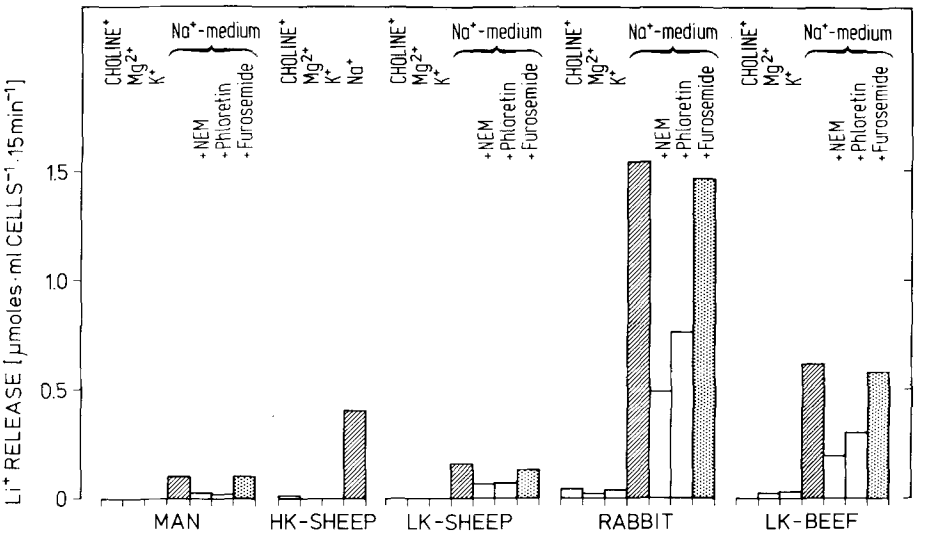

Fig. 4. Effects of cation composition of the medium, NEM, phloretin, and furosemide on Li^+ release. Erythrocytes from the same donor and animals used in the experiments shown in Fig. 1 were preloaded to contain 2 μmol Li^+/ml cells, washed, and resuspended in isotonic choline$^+$, Mg^{2+}, K^+ or Na^+ chloride media (0.1 mM ouabain, pH 7.4, 37 °C, hematocrit 3%). Li^+ release was measured over 1 hr with human and LK-sheep erythrocytes, over 30 min with bovine and HK-sheep cells, and over 15 min with rabbit erythrocytes, respectively, the values being normalized to 15 min in the figure for all species. In some cases (choline$^+$, Mg^{2+}, and K^+ media) the release lay below the level of graphical resolution. The NEM and phloretin concentrations were each 1 mM in the experiments with sheep erythrocytes, in all other cases 0.5 and 0.2 mM, respectively. The furosemide concentration was 1 mM

distribution (Donnan ratio $r = Na^+_e/Na^+_i = 0.65$). The Na^+-K^+ pump was inhibited by ouabain. In the absence of external Li^+, the cell Na^+ content remained almost constant, as to be expected from the electrochemical equilibrium prevailing under these conditions. However, when Li^+ was added externally, Na^+ was removed from the cells against an electrochemical gradient. Concomitantly, Li^+ accumulated inside the cells, Li^+ net uptake slightly exceeding Na^+ net release. Na^+ and Li^+ net movements decreased with time due to progressive displacement of Na^+ by Li^+ from the internal site of the Na^+-Li^+ countertransport system (*see also* Fig. 1).

The total Li^+ uptake in Fig. 5 is the sum of Li^+ uptake mediated by the Na^+-Li^+ countertransport system and a portion proceeding through a leak (*see* Fig. 6). If Li^+ uptake through the leak is subtracted from the total uptake (yielding the dotted line in Fig. 5), the value for

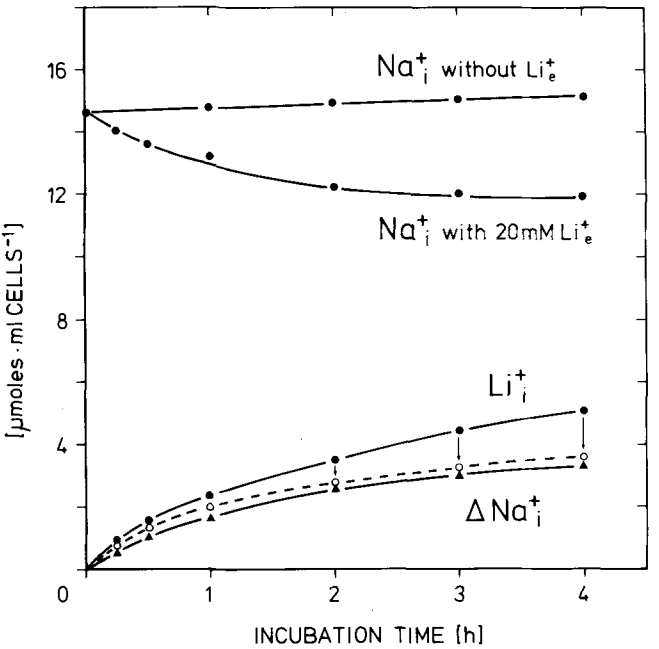

Fig. 5. Na^+ net release from rabbit erythrocytes induced by external Li^+. The cells were suspended in choline chloride media containing 15 mM Na^+ without and with 20 mM LiCl added (pH 7.4, 37 °C, 0.1 mM ouabain, hematocrit 3%). The Li^+-induced Na^+ net release presented at the bottom of the Figure (ΔNa^+_i) is the difference between the cellular Na^+ contents determined in the absence and presence of external Li^+. The portion of Li^+ uptake actually mediated by the Na^+-Li^+ exchange system is represented by the broken line. The latter is obtained from the curve describing the increase of cellular Li^+ content by subtracting Li^+ transport proceeding through the leak (*see* text)

Li^+ downhill and Na^+ uphill net movements become nearly identical. Na^+ and Li^+ are thus exchanged by the Na^+-Li^+ countertransport system with a stoichiometry of 1:1.

Li^+ Uptake through the Li^+ Leak

As demonstrated in Fig. 6 for the case of rabbit erythrocytes, the dependence of total ouabain-resistant Li^+ uptake on external Li^+ concentrations ranging up to 150 mM can be resolved graphically into a saturating and a linear component. The saturating component is the portion of Li^+ uptake mediated by the Na^+-Li^+ exchange system, the linear component operationally being ascribed to a leak. The latter amounted to 0.018 μmol Li^+ uptake/ml cells × hr per 1 mM increment in external

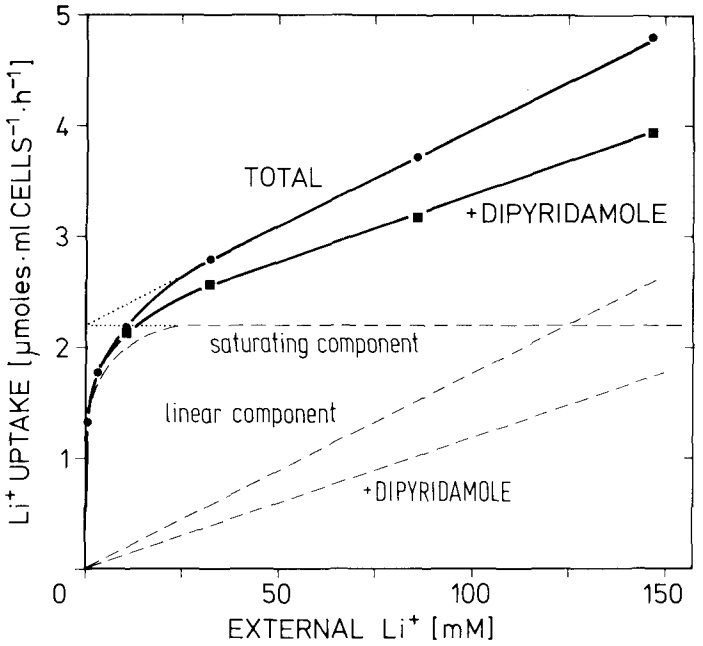

Fig. 6. Saturating and linear components of ouabain-resistant Li^+ uptake in rabbit erythrocytes. Li^+ uptake was determined over 1 hr on erythrocytes from the animal also studied in Fig. 5. The cells were suspended in media of different Li^+ concentration in the absence and presence of dipyridamole (0.05 mM). Choline chloride was replaced by LiCl (pH 7.4, 37 °C, hematocrit 3%, 0.1 mM ouabain). The Li^+ uptake is resolved graphically into a saturating and linear component (broken lines)

Li^+ for rabbit erythrocytes (Fig. 6). There are slight species differences in the leak, the values for human, LK-sheep, HK-sheep, and beef erythrocytes being 0.014 ± 0.002 ($n=6$), 0.0073 ± 0.0007 ($n=3$), 0.0063 ($n=1$), and 0.0061 ± 0.001 ($n=4$), respectively (mean values $\pm$ 1 SD from the number of donors or animals indicated by n). Dipyridamole (0.05 mM) inhibited the Li^+ leak by 30–60% in all four species (*see* Fig. 6 for the rabbit, *see also* Duhm & Becker, 1978*a*), the half maximum effect being observed with human erythrocytes at a dipyridamole concentration of 2 μM, i.e., a concentration which also induces 50% inhibition of anion exchange (Deuticke, 1970).

Species Differences in Na^+-Li^+ Exchange

The dependencies of Li^+ uptake on the external Li^+ concentration of erythrocytes from an individual LK-sheep, rabbit, and LK-beef are

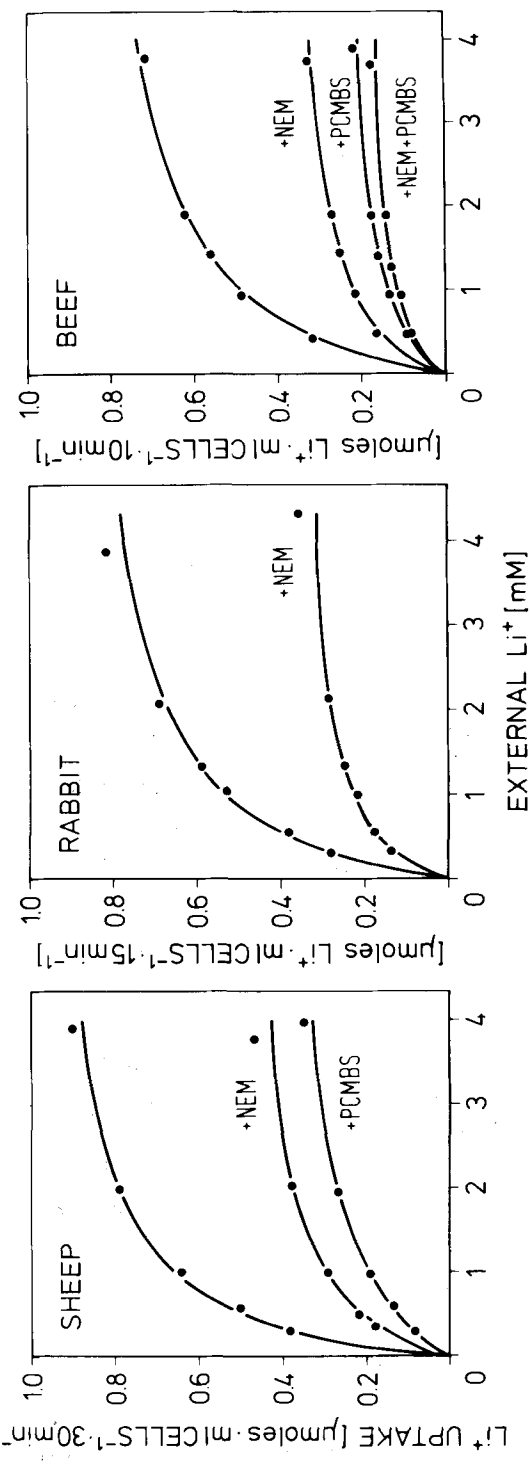

Fig. 7. Affinity of the Na^+-Li^+ exchange system of LK-sheep, rabbit, and beef erythrocytes for external Li^+. The erythrocytes were incubated for the time intervals given on the ordinates at various Li^+ concentrations in choline chloride media (0.1 mM ouabain, pH 7.4, hematocrit 3%). The NEM concentration was 0.5 mM for rabbit and beef, and 1 mM for sheep erythrocytes. The PCMBS concentration was 25 μM for sheep and 5 μM for beef erythrocytes. The curves were fitted to the points similarly to the method described in the legend of Fig. 3*b* with apparent K_m values (mM) of external Li^+ for sheep of 0.5 (control), 0.7 (NEM), and 1.2 (PCMBS), for rabbit of 0.7 (control) and 0.6 (NEM), and for beef of 0.8 (control, NEM, and PCMBS) and 0.6 (NEM+PCMBS), respectively. The slightly elevated K_m value for sheep erythrocytes found in the presence of PCMBS results from a PCMBS-induced increase of the Li^+ leak, the sheep being particularly sensitive to PCMBS in this respect

compared in Fig. 7. Li^+ uptake tended to saturate at 4 mM external Li^+ in all species, despite the pronounced differences in the rates of transport (note the designations on the ordinates in Fig. 7). NEM reduced Li^+ uptake by 50–60% at all Li^+ concentrations in each case. PCMBS was an even more potent inhibitor in sheep and beef erythrocytes, but did not impede Li^+ uptake by rabbit erythrocytes. Combination of NEM and PCMBS only slightly increased the inhibitory effect above the value obtained with PCMBS alone. The reduction of Li^+ uptake by these SH-reagents results from a decrease in maximum transport rate, whilst the affinities for external Li^+ are not essentially altered (*see* legend of Fig. 7).

Values of the apparent kinetic parameters K_m and V_{max} of Li^+ uptake, derived from experiments such as shown in Fig. 7, are presented in Table 1. Evidently, the maximum transport rates show large interspecies (and interindividual) differences, whereas the apparent K_m values for external Li^+ are similar among the species (and individuals).

A further difference between the species concerns the susceptibility of Na^+-Li^+ exchange to inhibitors. Phloretin and NEM retard Na^+-Li^+ exchange in all of the four species, but PCMBS is only effective on beef and sheep erythrocytes (Table 2). Furosemide and quinidine were found to be weak inhibitors of Li^+ uptake at a concentration of 1 mM in the four species as was 0.5 mM ethacrynic acid (Table 2). At higher concentrations, furosemide blocked Li^+ uptake markedly in human erythrocytes, whilst only a slight increase in inhibitory potency was observed with rabbit and bovine red cells (Fig. 8). Some differences in sensitivity towards NEM, PCMBS, and phloretin are evidenced by the

Table 1. Inter- and intra-species comparison of the apparent maximum Li^+ uptake (V_{max}) and Li^+ affinity of the external site (K_m) of the Na^+-Li^+ exchange system[a]

Specimen No.	Man		HK-Sheep		LK-Sheep		Rabbit		LK-Beef	
	V_{max}	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}	K_m
1	0.29	1.6	0.94	0.5	2.0	0.5	3.6	0.7	9.1	0.6
2	0.25	1.4			1.9	0.5			5.5	0.7
3	0.18	1.6			1.2	0.8			5.3	0.8
4	0.09	1.8								

[a] The values are given in μmol/ml cells × hr and mM, respectively. The data were obtained in experiments such as shown in Fig. 7. The values for human erythrocytes are taken from Duhm and Becker (1977*b*) and are determined in choline chloride media containing 10 mM Na^+, whereas the choline media used for the other species were free of added Na^+.

Table 2. Species comparison of cellular cation contents, Li^+ uptake, and its inhibition by various agents[a]

		Na^+	K^+	Li^+ uptake	Percent inhibition of Li^+ uptake					
		(μmol/ml cells)		(μmol/ml cells × hr)	NEM	PCMBS	Phloretin	Furosemide (1 mM)	Ethacrynic acid (0.5 mM)	Quinidine (1 mM)
Human		9±1	96±4 (40)	0.12±0.04 (40)	47±8 (6)	0[b]	70±8 (10)	20±4 (5)	22±5 (3)	11±13 (3)
Sheep	HK	10±1	84±3 (4)	0.76±0.06 (4)	47 (1)	37 (1)	53 (1)	14 (1)	0 (1)	6 (1)
	LK	89±4	12±4 (13)	1.16±0.24 (13)	53±2 (10)	58±2 (9)	68±6 (9)	5±6 (6)	2±3 (6)	7± 3 (6)
Rabbit		11±5	100±6 (15)	2.60±0.34 (15)	59±3 (7)	0[b]	48±7 (5)	3±1 (4)	3±4 (3)	12 (1)
Beef	LK	62±5	21±5 (31)	3.50±1.52 (31)	59±4 (20)	76±6 (28)	68±9 (6)	11±7 (4)	1±3 (4)	26±14 (4)

[a] NEM, PCMBS, and phloretin were applied in concentrations which induce the optimum effect in each species (0.5 mM NEM, 5 μM PCMBS, and 0.2 mM phloretin with human, rabbit, and beef erythrocytes, 1 mM each of NEM and phloretin and 25 μM PCMBS with sheep erythrocytes). Li^+ uptake was determined in choline media (2 mM Li^+, pH 7.4, 37 °C, hematocrit 3%, 0.1 mM ouabain). Mean values ±1 SD from the number of donors and animals given in parentheses, except for HK-sheep where the numbers in parentheses are numbers of experiments with red cells of the one HK-sheep available.

[b] No inhibition at 1–10 μM PCMBS; at 25–100 μM PCMBS, increasing leak.

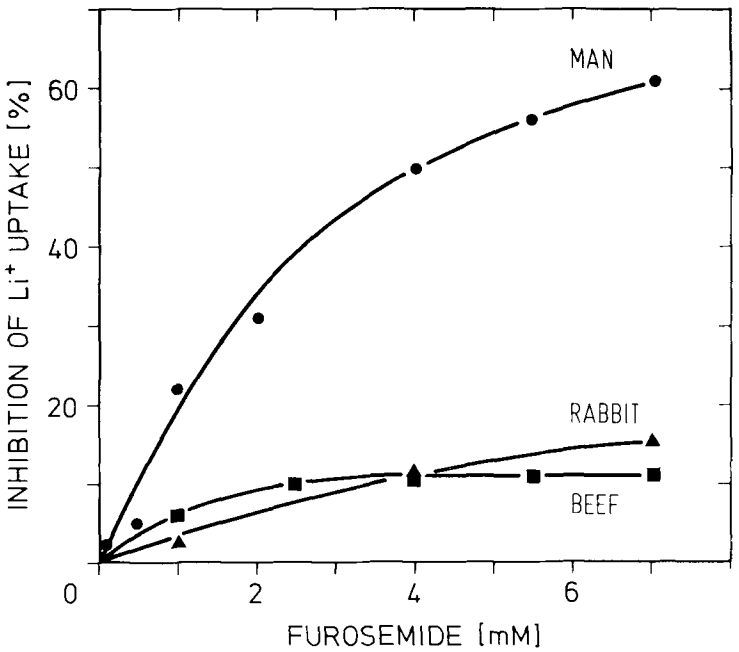

Fig. 8. Inhibition of Li^+ uptake by furosemide. The uptake was measured in choline chloride media containing 2 mM Li^+ (37 °C, pH 7.4, 0.1 mM ouabain, hematocrit 3%). The uninhibited values were 0.21 μmol Li^+ uptake/ml cells × hr with human erythrocytes (donor U.L.) and 0.75 and 1.75 μmol Li^+ uptake/ml cells × 15 min for rabbit and beef erythrocytes, respectively

different amounts of reagents required per ml of cells to achieve near maximum inhibition (*see* legend of Table 2).

The inhibitory effects of the various agents are given in Table 2 on a percentage basis. As can be assessed from the relatively small standard deviations, the percentage inhibition induced each by NEM, PCMBS, and phloretin was very similar among the individuals of one species, independent of the individual rate of transport.

Interindividual Differences in Na^+-Li^+ Exchange

Na^+-Li^+ countertransport also varies among individual members of one and the same species. If the Li^+ leak (dotted lines in Fig. 9) is subtracted from the Li^+ uptake values displayed in Fig. 9, the data demonstrate that the highest and lowest values of Na^+-Li^+ exchange differed by a factor of about seven among human and by a factor of

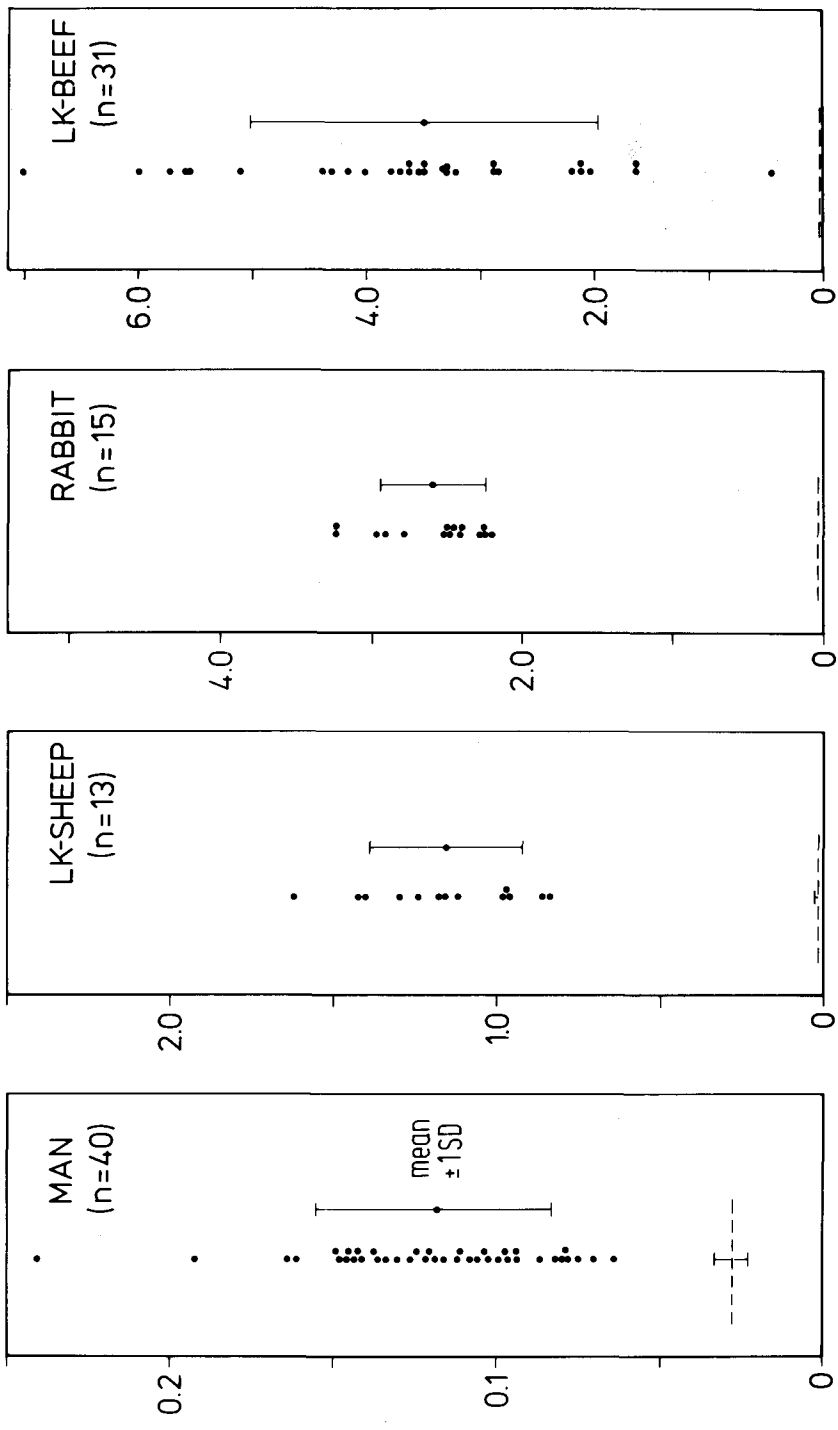

Fig. 9. Interindividual variations of Li^+ uptake among erythrocytes of four mammalian species. Note the differences in the ordinate scales. Li^+ net uptake was determined in choline media containing 2 mM Li^+, experimental conditions as described in the legend of Fig. 1. The uptake period was 1 hr for human, 30 min for sheep, and 15 min for rabbit and beef erythrocytes, the values being normalized to 1 hr in the figure for all species. The broken lines indicate Li^+ *transport proceeding through the leak* (assessed from data such as shown in Fig. 6, *see* text)

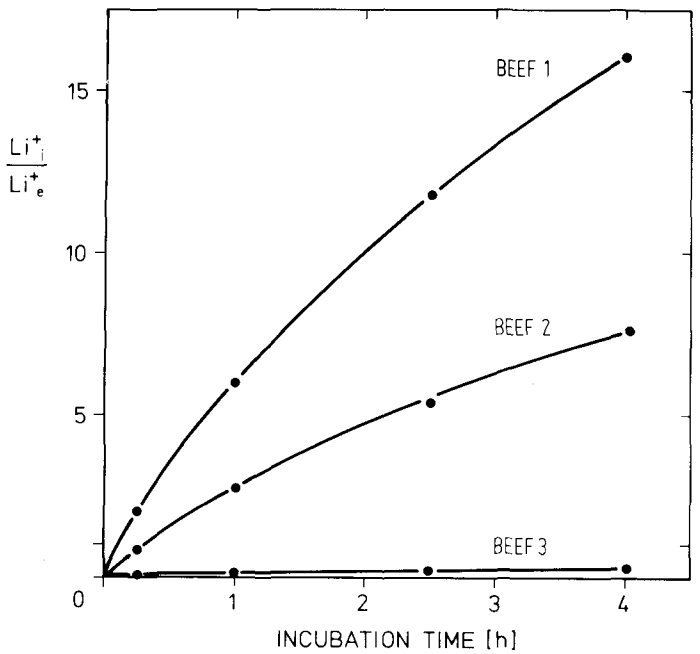

Fig. 10. Development of the ratio of internal Li^+ (μmol/ml cells) to external Li^+ (mM) with time in beef erythrocytes with different activities of the Na^+-Li^+ exchange system. The erythrocytes were incubated in choline chloride media initially containing 0.5 mM Li^+ (hematocrit 3%, 37 °C, pH 7.4, 0.1 mM ouabain). The individual activities of the exchange system, as assessed from Li^+ uptake in choline media containing 2 mM Li^+ (*see* Fig. 9), were 1.75, 0.87, and 0.11 μmol Li^+ uptake/ml cells × 15 min, and the Na^+ contents were 65, 65, and 61 μmol/ml cells after 4 hr of incubation for the beef No. *1*, *2* and *3*, respectively

sixteen among bovine erythrocytes. The rate of Na^+-Li^+ exchange was about 200 times greater in the fastest bovine than in the slowest human red cell specimen. The fluctuations among LK-sheep seem to be lower, only a twofold difference between the extreme values having been observed in this species. Rabbits display the lowest variability.

The importance of the individual activity of the Na^+-Li^+ countertransport system for the establishment of an outwardly directed Li^+ gradient by erythrocytes is shown in Fig. 10 for the slowest and fastest bovine red cell specimen from Fig. 9 and for one with medium activity. The cells were suspended in choline chloride media at an external Li^+ concentration of 0.5 mM. The ratio of cellular Li^+ content to external Li^+ concentration, Li^+_i/Li^+_e, increased to a value of 16 within 4 hr in the case of the specimen with the highest Na^+-Li^+ exchange capacity, whereas in the sample with the lowest countertransport activity the ratio

was still far below one after 4 hr. A steady-state was not attained within this time. The Na^+ ratio, a measure of the driving force for Li^+ uphill transport into the cells, was about 600 after 4 hr for the erythrocytes of the beef No. 3, i.e., much higher than in the two other beef (230 and 270, Nos. *2* and *1* in Fig. 10). The Li^+ leak was not significantly different among the red cell specimens *1*, *2*, and *3* (0.006, 0.007, and 0.007 μmol Li^+ uptake/ml cells × hr per 1 mM Li^+ concentration difference, respectively, values estimated from experiments such as shown in Fig. 6).

pH-Dependence of Li^+ Uptake

The pH-dependence of Li^+ uptake by human and bovine erythrocytes is compared in Fig. 11 with the pH dependence of ouabain-resistant Na^+-Na^+ exchange in bovine erythrocytes. The transport rates are nor-

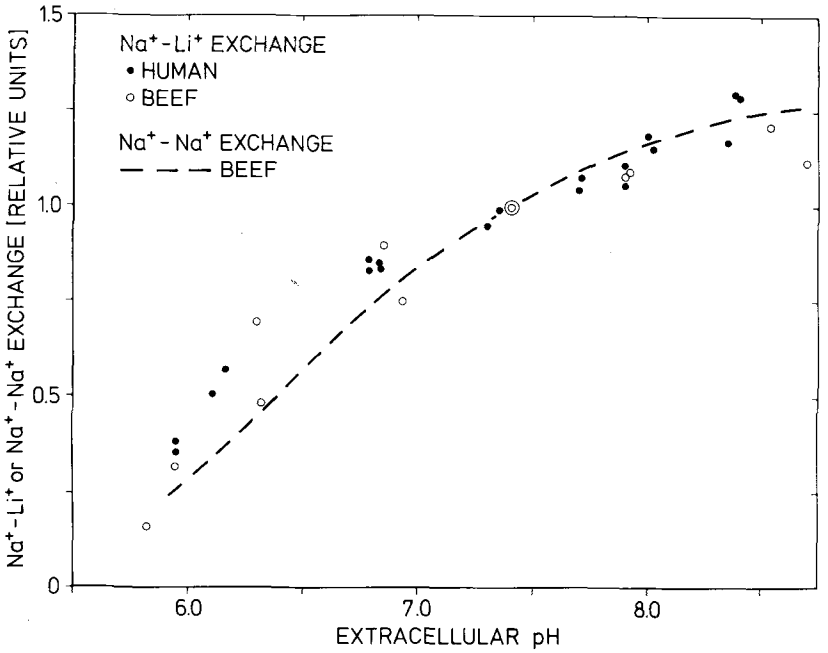

Fig. 11. Comparison of the pH dependence of Na^+-Li^+ exchange in human (●) and beef (○) red cells with that of Na^+-Na^+ exchange in beef erythrocytes (broken line, taken from Fig. 11 of Motais (1973)). Na^+-Li^+ exchange activity was assessed by measurements of Li^+ uptake from choline chloride media containing 2 mM Li^+ with erythrocytes of the donor J.D. and two beef (0.1 mM ouabain, 37 °C, hematocrit 3%). The incubation times were 1 hr for human and 10 min for beef erythrocytes. All data are expressed as a fraction of the rate observed at pH 7.4 (0.2 μmol Li^+/ml cells × hr for the human, 0.74 and 0.93 μmol Li^+/ml cells × 10 min for the two beef red cell specimens, and 7 μmol Na^+/ml cells × hr for Na^+-Na^+ exchange of the beef erythrocytes studied by Motais (1973)

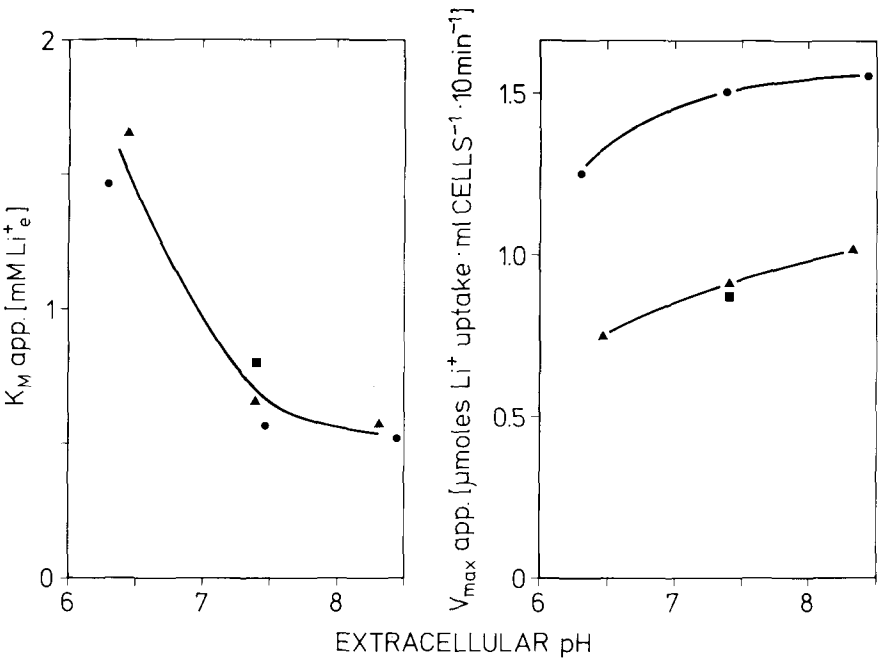

Fig. 12. pH-Dependence of the kinetic parameters of the Na^+-Li^+ exchange system of bovine erythrocytes. The apparent kinetic constants K_m for external Li^+ and V_{max} for Li^+ uptake were determined as in Fig. 7 at the extracellular pH values indicated. Different symbols refer to individual animals

malized in each case to a fraction of the rate at pH 7.4. Clearly, Na^+-Na^+ exchange in beef and Na^+-Li^+ exchange in human and beef erythrocytes display a rather similar pH-dependence. The Li^+ uptake rates increased about 3.5-fold upon elevation of the extracellular pH from 6 to 8.5 in both types of erythrocytes, despite the marked differences in the absolute rates of transfer (*see* legend of Fig. 11). PCMBS inhibited Li^+ uptake in beef erythrocytes by about 70% at all pH values (data not shown).

The pH-dependence of Li^+ uptake presented in Fig. 11 arises largely from a reduction in Li^+ affinity of the external transport site with rising hydrogen ion concentration. This is demonstrated for two separate beef in Fig. 12. Lowering the pH from 8.5 to 6 leads to an about threefold increase in the apparent K_m value for external Li^+, whereas the apparent maximum transport capacity drops only slightly. The drop in apparent V_{max} need not reflect a real pH dependence of the maximum transport capacity, but could be the result of the lower cellular Na^+ concentration at low pH (due to cell swelling) and/or of a reduction in Na^+ affinity of the internal site of the transport system, analogous to that observed for Li^+ at the external site.

Discussion

Species Differences in Na^+-Li^+ Exchange

Considerable species differences in the rate of Na^+-Li^+ exchange across the red cell membrane are observed, the mean rates of Li^+ uptake by the exchange system increasing about 30-fold in the order human < HK-sheep < LK-sheep < rabbit < LK-beef erythrocytes (Figs. 1 and 9, Tables 1 and 2). These differences in Li^+ uptake are largely caused by species-characteristic differences in the maximum activity of the Na^+-Li^+ exchange system (*see* Tables 1 and 2). The affinities of the transport system for Li^+ and Na^+ are rather similar among the species. Differences in cellular Na^+ content contribute to the species variability, but account only for a factor of about two between HK and LK species.

The relative rates of Na^+-dependent Li^+ release among the species are not identical to those noted for Li^+ uptake. For instance, the sequences rabbit < beef and HK-sheep < LK-sheep observed with Li^+ uptake (Table 2) are reversed in the case of Li^+ release (Fig. 4). These inversions can be ascribed to the more effective competition of internal Na^+ with internal Li^+ to be released in those erythrocytes with high Na^+ contents, i.e., in erythrocytes of LK-sheep and beef (*see* Table 2).

Interindividual Differences in Na^+-Li^+ Exchange

The variability among individuals of one species is also primarily due to differences in the maximum transport capacity of the Na^+-Li^+ exchange system. This interindividual variability is most marked in bovine and human erythrocytes, less pronounced in sheep, and seemingly absent in rabbit red blood cells (Fig. 9). Very similar K_m values for Li^+ were found in samples with different maximum capacity (Table 1).

Another factor contributing to the intraspecies variability is the cellular Na^+ concentration (*see* Fig. 3). In the case of the genetic dimorphism in sheep, the higher internal Na^+ concentration in LK-sheep erythrocytes can account for the about twofold faster rate of Li^+ uptake as compared to that found in HK-sheep (Table 2). The differences in cellular Na^+ concentration among the LK-beef (and LK-sheep) examined contribute only minimally to the interindividual variability because in these cells the Na^+ concentration is several times higher than the K_m value of about 10 mM for internal Na^+. In human and rabbit erythrocytes, how-

ever, the internal Na^+ concentration is in the vicinity of the half saturating value. Hence, the interindividual variability of red cell Na^+ content in man and rabbit must be of importance in determining the individual rate of Li^+ uptake and the steady-state Li^+ distribution across the red cell membrane, respectively. Li^+ uptake by the rabbit erythrocytes studied was indeed highly correlated to the intracellular Na^+ concentration. Although the corresponding relation for human erythrocytes was overruled by the interindividual variability in transport capacity, the red cell Na^+ concentration needs to be considered as a variable in studies concerning the inheritance of the individual activity of the human red cell Na^+-Li^+ countertransport system.

Similar to the situation in human erythrocytes (Duhm & Becker, 1977*b*; Greil *et al.*, 1977; Sarkadi *et al.*, 1978), the differences in the *in vitro* Li^+ distribution across the bovine red cell membrane are caused by individually different activities of the Na^+-Li^+ exchange system (Fig. 10), and do not arise from differences in the Li^+ leak, as suggested by Funder and Wieth (1978).

Relation of the Ouabain-Resistant Na^+-Li^+ Exchange System to other Cation Transport Systems

Na^+-Li^+ exchange is found to be only slightly inhibited by furosemide and ethacrynic acid (*see* Table 2 and Fig. 4) when applied in concentrations (0.5–1 mM) known to completely block the "second", ouabain-resistant Na^+ transport system(s) (Hoffman & Kregenow, 1966; Garrahan & Glynn, 1967; Lubowitz & Whittam, 1969; Dunn, 1973; Wiley & Cooper, 1974; Beaugé, 1975; Sachs, Knauf & Dunham, 1975; McManus & Schmidt, 1978). Much higher concentrations of furosemide were required in our experiments to markedly reduce Na^+-Li^+ countertransport in human erythrocytes (Fig. 8), and the drug remained only slightly effective in the other species, even at a concentration of 7 mM. Quinidine (1 mM) had also only a small effect on Li^+ transport in the four species (Table 2). Similarly, Motais and Sola (1973) and Frazer, Mendels and Brunswick (1977) found 1 mM furosemide to be without effect on Na^+-Na^+ exchange in beef and Na^+-Li^+ exchange in human erythrocytes. These results indicate that the Na^+-Li^+ exchange system is not identical with the (or one of the) "second" Na^+ transport system(s). In contrast to these findings, Pandey *et al.* (1978) and Sarkadi *et al.* (1978) reported that 1 mM furosemide or quinidine fully block the Na^+-Li^+ exchange system of human erythrocytes. The reason for the discrepancy in results is not obvious, but it is to be noted that

the experiments reported by Pandey *et al.* (1978) and Sarkadi *et al.* (1978) were performed on erythrocyte specimens of much lower Na^+-Li^+ exchange activity than that found in human red cells used for the present study.

It has been suggested by Gunn (1978) that Na^+-Li^+ exchange is the result of an obligatory exchange of $NaCO_3^-$ for $LiCO_3^-$ ion pairs, mediated by the anion exchange system. This proposal seems rather unlikely because Na^+-Li^+ exchange as such is not affected by complete removal of CO_3^{2-}, the anion required for the formation of the ion pairs considered (Becker & Duhm, 1978; Duhm & Becker, 1978*b*; Funder *et al.*, 1978). Furthermore, inhibitors of anion exchange do not inhibit Na^+-Li^+ exchange (Duhm *et al.*, 1976; Duhm & Becker, 1977*a*, *b*, 1978*a*, *b*; *see also* Fig. 3).

Another possibility is that ouabain-resistant Na^+-Li^+ exchange might be mediated by an ouabain-resistant function of the Na^+-K^+ pump. Indeed, part of the ouabain-sensitive Li^+ uptake by human and rat erythrocytes has been found to be insensitive to oligomycin (*see also* Duhm & Becker, 1977*a*), a further inhibitor of the Na^+-K^+ pump, but dependent on cellular inorganic phosphate (J. Duhm, *unpublished results*). However, the large interindividual differences in Na^+-Li^+ exchange observed with human erythrocytes are difficult to reconcile with this idea. No differences in ouabain-sensitive Li^+ uptake have been observed among human erythrocytes of threefold differing exchange capacity (Duhm & Becker, 1977*b*), and no drastic variability in Na^+-K^+ pump activity has been reported in the literature for normal human erythrocytes. In addition, HK- and LK-sheep erythrocytes, which show different kinetic properties and a fivefold difference in Na^+-K^+ pump activity at physiological cation concentrations (Tosteson & Hoffman, 1960; Lauf, 1975), exhibit only a small difference in Na^+-Li^+ exchange, this being fully accounted for by the respective cellular Na^+ concentrations. Finally, LK-beef red cells and rabbit erythrocytes are cells with similarly high rates of Na^+-Li^+ exchange, but markedly divergent Na^+-K^+ pump activities (*see* Table 3). A relation of Na^+-Li^+ exchange to the functioning Na^+-K^+ pump thus seems unlikely. It cannot be excluded, however, that during maturation of the erythrocytes in the bone marrow (*see* Kirk, Lee & Tosteson, 1978) some Na^+-K^+ pump molecules are altered (concomitant with a loss of Na^+-K^+ pump activity), so as to mediate a ouabain-resistant Na^+-Na^+ and Na^+-Li^+ exchange which is not inhibited by external K^+, instead of the ouabain-sensitive Na^+-Na^+ (Na^+-Li^+?) exchange of the normal pump which is sensitive to external K^+ (Garrahan & Glynn, 1967; Sachs *et al.*, 1975).

Table 3. Relative activities of cation transport systems in human, sheep, rabbit, and beef erythrocytes

Species	Red cell type	Na^+-Li^+ exchange[a] (ouabain-resistant)	Na^+-Na^+ exchange[b] (ouabain-resistant)	Na^+-K^+ pump[c] (ouabain-sensitive)
Man	HK	1	1[d]	1[h]
Sheep	HK	6.3	5.8[e]	0.4[e]
	LK	9.8	8.2[e]	0.1[e]
Rabbit	HK	22	13[f]	1.8[h]
Beef	LK	29	21[g]	0.2[h]

[a] Mean rates taken from Table 2; $1 \triangleq 0.12$ μmol Li^+ uptake/ml cells × hr.
[b] $1 \triangleq 0.6$ μmol/ml cells × hr.
[c] $1 \triangleq 1.5$ μmol K^+ uptake/ml cells × hr.
[d] Estimated from Lubowitz and Whittam (1969) and Sachs (1971).
[e] Taken from Tosteson and Hoffman (1960).
[f] Estimated from Rettori *et al.* (1969) and Villamil and Kleeman (1969).
[g] Estimated from Motais (1973) and Funder and Wieth (1978).
[h] Taken from Kirk (1977).

A high parallelism is to be found between the rates of Na^+-Li^+ exchange and ouabain-resistant Na^+-Na^+ exchange in the four species examined (Table 3). In addition, ouabain-resistant Na^+-Na^+ and Na^+-Li^+ exchange exhibit the following common properties:

Both exchange modes are tightly coupled with a stoichiometry of 1:1. This has been shown for Na^+-Li^+ exchange in rabbit (Fig. 5), beef (Funder & Wieth, 1978) and human erythrocytes (Sarkadi *et al.*, 1978), and for ouabain-resistant Na^+-Na^+ exchange in beef (Motais, 1973), sheep (Tosteson & Hoffman, 1960) and possibly also in human erythrocytes (Lubowitz & Wittam, 1969; Sachs, 1971).[1]

The affinity of the external site for Na^+ is low in the two exchange modes ($K_m = 40$–50 mM in beef (Motais, 1973) and in LK- and HK-sheep (Tosteson & Hoffman, 1960) and 25–50 mM in human erythrocytes (Duhm & Becker, 1977*b*; Sachs, 1971; Sarkadi *et al.*, 1978)).

[1] Considerable confusion exists in the literature concerning the actual presence of a ouabain-resistant Na^+-Na^+ exchange in human erythrocytes. One reason for the discrepancy in the results discussed in detail by Sachs *et al.* (1975) may be that human erythrocytes with a relatively high activity of the exchange system have been studied by those authors who found the exchange (e.g., Lubowitz & Whittam, 1969; Sachs, 1971), whereas the other authors incidentally may have examined red cells with a very low or almost absent activity of the system (e.g., Dunn, 1973; Beaugé, 1975). Another possibility might be that some of the maneuvers applied to vary the intracellular concentrations of Na^+ or ATP may have interferred with the exchange system. As will be discussed in detail in a forthcoming paper, iodoacetamide, for instance, which is often used to reduce the cellular ATP content, can induce an irreversible blockade of the ouabain-resistant Na^+-Li^+ exchange system of human and bovine erythrocytes (as does NEM) when either Na^+ or Li^+ is present in the incubation medium.

The affinity of the internal site is 3–5 times higher and again similar for Na^+-Na^+ and Na^+-Li^+ exchange in beef ($K_m = 10$ mM, Motais, 1973) and human erythrocytes ($K_m = 6$–10 mM, Fig. 3, Sachs, 1971; Sarkadi *et al.*, 1978).

Na^+ and Li^+ compete with one another when present at the same side of the membrane in erythrocytes of rabbits (Fig. 2), sheep (compare LK- and HK-sheep in Fig. 4), man (Duhm & Becker, 1977*a, b*, 1978*b*; Sarkadi *et al.*, 1978) and beef (Funder & Wieth, 1978).

The pH-dependencies of ouabain-resistant Na^+-Na^+ and Na^+-Li^+ exchange are virtually identical (Fig. 11).

Further similarities are the inhibition of both ouabain-resistant Na^+-Na^+ and Na^+-Li^+ exchange by NEM in human, beef, and sheep erythrocytes, and additionally by PCMBS in beef and sheep, but not in human erythrocytes (*see* Table 2 and Fig. 7, and Motais & Sola, 1973). As assessed from Table 2 of Lubowitz and Whittam (1969), ouabain-resistant Na^+-Na^+ exchange in human erythrocytes shows an interindividual variability corresponding to that found for Na^+-Li^+ exchange in Fig. 9. Inspection of the data of Motais (1973) reveals that Na^+-Na^+ exchange in beef erythrocytes varies by a factor of at least two.

This high mutuality in properties strongly suggests that *a transport mechanism exists in the four species which can mediate ouabain-resistant exchange of either Na^+ for Na^+, Na^+ for Li^+, or Li^+ for Li^+.*

Evidence for ouabain-resistant Na^+-Na^+ exchange has been obtained also in other organs, including smooth (Brading, 1975) and skeletal muscle (Keynes & Swan, 1959; Keynes & Steinhardt, 1968; Beaugé & Sjodin, 1968) and Ehrlich ascites tumor cells (Mills & Tupper, 1975). A Na^+-Li^+ countertransport mechanism with a K_m value for external Na^+ comparable to that of the red cell system has been demonstrated in frog skeletal muscle (Smith, 1974). Hence, it appears not unlikely that other organs possess a ouabain-resistant system exchanging Na^+ for Na^+ or Na^+ for Li^+, similar to that described in the present paper. If this were so, several transport processes presently not understood would find an explanation, e.g., Li^+ extrusion against an electrochemical gradient from nerve (Thomas, Simon & Oehme, 1975) and muscle cells (Smith, 1974), and Li^+ uphill transport across epithelia (Reinach, Candia & Siegel, 1975).

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