Pig Chow (Ralston Purina Co., St. Louis, MO, USA), and neither the chow nor the drinking water was supplemented with any additional sodium.

At the times indicated below, 20-ml blood samples were drawn into heparinized syringes and red blood cell water and electrolyte contents measured within 1 hour. Water content was determined in duplicate by centrifuging 0.4-ml aliquots of whole blood for 5 minutes at 9000 g in a Beckman Microfuge B (Beckman Instruments Inc., Fullerton, CA, USA). The supernatant and the uppermost layer of cells were completely aspirated and discarded, the remaining cell pellet was weighed, and the sample was dried for 48 hours at 90 °C. The dried cell pellet was weighed, and cell water content was determined as the difference between wet and dry weights. No correction was made for trapped plasma. Cell electrolyte levels were determined after centrifuging the blood sample, removing and discarding the plasma and buffy coat, and washing the cells at least four times in an ice-cold washing solution consisting of (mM) 75 MgCl₂, 85 sucrose, 10 tris(hydroxymethyl)aminomethane morpholinepropanesulfonic acid (TRIS-MOPS; pH 7.4 at 4 °C). After the fourth wash, supernatant sodium concentration was determined by atomic absorption spectrophotometry (Perkin-Elmer Model 2380 AA Spectrophotometer, Perkin-Elmer Corporation, Norwalk, CT, USA) and, if greater than 30 μM, washing was repeated until the sodium concentration was below that level. A 200-μl aliquot of washed cells was lysed in 10 ml of 5% tricarboxylic acid, centrifuged, and stored for subsequent determination of sodium and potassium.

Five milliliters of the washed cells was transferred to a 250-ml Erlenmeyer flask containing 20 ml of a lithium loading solution consisting of (mM) 150 LiCl, 10 TRIS-MOPS (pH 7.4 at 37 °C), 10 glucose and incubated for 3 hours at 37 °C in a shaking water bath. After incubation, the suspension was centrifuged for 5 minutes at 1000 g, the supernatant was discarded, and the cells were washed free of external lithium with washing solution to which 0.1 mM ouabain had been added. The cells, now loaded with lithium, were suspended at an approximate hematocrit of 50% in this solution, and the hematocrit was determined in duplicate. A 200-μl aliquot was lysed in 10 ml of 5% trichloroacetic acid for determination of cation contents. Two 1.6-ml aliquots were added to 10 ml of either a magnesium chloride solution (MS) of (mM) 75 MgCl₂, 85 sucrose, 10 TRIS-MOPS (pH 7.4 at 37 °C), 0.1 ouabain, 10 glucose or a sodium chloride solution (SS) of (mM) 150 NaCl, 10 TRIS-MOPS (pH 7.4 at 37 °C), 0.1 ouabain, 10 glucose. For the choline and potassium media, equimolar substitution of the appropriate chloride salt was made for NaCl in SS. Phloretin (Sigma Chemical Co., St. Louis, MO, USA) was prepared as a 100 mM solution in dimethylsulfoxide and added to the desired final concentrations (0.2–1.0 mM). For the bumetanide experiments, bumetanide was prepared as a 10 mM solution in dimethylsulfoxide and added to the MS and SS efflux solutions to produce a final concentration of 0.1 mM. After thorough mixing, 1.5-ml aliquots of each of the suspensions were pipetted into six tubes. All tubes were incubated at 37 °C in a shaking water bath, and duplicates were removed at 20, 40, and 60 minutes, iced for 2 minutes, and then centrifuged at 1000 g for 5 minutes. Supernatants were carefully removed and assayed for lithium content by atomic absorption spectrophotometry using standards prepared in MS (used for MS and choline chloride solution), potassium chloride solution, or SS as appropriate. Effluxes were determined by linear regression fits of lithium concentration versus time, and correlation coefficients (r) of greater than or equal to 0.98 were considered technically satisfactory. The difference between the effluxes in MS and SS was taken as the rate of SDLE and is expressed as micromoles of lithium per liter of cells per hour.

All data are reported as mean ± SEM. Differences between the groups were analyzed by paired t test (two-tailed), and significance was accepted at the p < 0.05 level.

Results

Lithium Transport in Pig Erythrocytes

Pig erythrocytes suspended at a nominal hematocrit of 5% in the lithium loading solution accumulated lithium linearly throughout the 3 hours of incubation. Final lithium concentrations achieved averaged about 5 mmol/L cells (see Table 2). During lithium loading, cell sodium and potassium declined from values obtained in fresh, washed cells, but total cell cation content was not significantly altered (Tables 1 and 2).

Lithium efflux from lithium-loaded cells into isotonic sodium-free media (MS, choline chloride solution, and potassium chloride solution) was linear, and efflux rates were not significantly different in the three sodium-free media (Figure 1). Efflux rates for lithium

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**Figure 1.** Effect of external cation substitution and phloretin on lithium efflux from lithium-loaded pig red cells. Efflux into magnesium chloride solution (MS) defined as 100%. CS = choline chloride solution; KS = potassium chloride solution; SS = sodium chloride solution. Bars represent means ± SEM for cells from four animals.
in the sodium-free media showed approximately threefold interindividual variability (range of lithium effluxes for MS: 418–1194 µmol/L cells/hr), and rate coefficients were some four to eight times higher than we have previously observed in either humans or sheep of low potassium and high potassium phenotypes (unpublished observations, 1984). For any individual animal, lithium efflux was invariably more rapid in the sodium-rich than in any of the sodium-free media. Although the efflux into SS was essentially linear over the time course employed (1 hour), in some experiments, particularly in those animals with the highest efflux rates, the late time point showed a downward deviation from linearity (i.e., efflux showed a tendency to slow slightly). Since calculated efflux rates were derived from linear regression analyses of external lithium concentrations, lithium efflux rates reported for the sodium medium are probably slight underestimates of initial efflux rates in some instances.

The countertransport inhibitor phloretin decreased lithium efflux into SS (see Figure 1). Total lithium efflux was inhibited by 44.3 ± 8.4% (mean ± SEM, n = 4) by 1.0 mM phloretin. The cotransport inhibitor bumetanide (0.1 mM) had no significant effect on lithium efflux into MS or SS.

### Effects of Deoxycorticosterone Acetate—Salt Treatment

Control and DOCA-treated pigs demonstrated similar weight gains over the 3 weeks of treatment (11.2 ± 0.8 vs 11.4 ± 0.7 kg, mean ± SEM, control vs DOCA). Mean arterial pressure was 110.2 ± 2.3 mm Hg in controls and 107.2 ± 3.1 mm Hg in DOCA-treated animals before pellet implantation. Blood pressure rose progressively in the DOCA-treated pigs and reached levels of 135.6 ± 7.4 mm Hg after 3 weeks (Figure 2). Blood pressure did not change appreciably in the control animals and averaged 110.8 ± 4.2 mm Hg after 3 weeks. The magnitude of the blood pressure rise was similar to that observed in earlier studies of this model.13, 14
Red blood cell sodium content increased in DOCA-treated pigs compared with controls (see Table 1). The maximal difference was reached at 10 days (5.87 ± 0.19 vs 7.17 ± 0.25 mmol/L cells, controls vs DOCA), and the increase was sustained for the duration of the study. Cell potassium levels showed an upward trend for both groups of animals, but differences between the groups were not statistically significant. Red cell water content was increased by a significant amount in DOCA-treated animals only on Day 5 (p < 0.05).

The level of red cell lithium achieved after loading was not significantly different between control and DOCA-treated animals before or at any time after implantation (see Table 2). Before implantation, net lithium effluxes from lithium-loaded cells were not significantly different in either MS (647 ± 70 vs 831 ± 410 μmol/L cells/hr, mean ± SEM, control vs DOCA) or SS (925 ± 96 vs 1096 ± 57, control vs DOCA), and SDLE was comparable (278 ± 41 vs 265 ± 53, control vs DOCA). Following implantation, SDLE rose progressively in DOCA-treated pigs but not in controls (Figure 3), and the difference was significant by Day 3 (344 ± 60 vs 455 ± 68, p < 0.05, control vs DOCA). The difference between the groups was maximal on Day 20 (256 ± 81 vs 648 ± 60 μmol/L cells/hr, p < 0.01, control vs DOCA).

As may be appreciated in Table 2, the rise in SDLE in the DOCA-treated animals was concurrent with a fall in lithium efflux into the sodium-free MS. Lithium efflux into SS, which represents the sum of SDLE and passive leak, did not change significantly in either group.

Discussion

These studies demonstrate the existence of a sodium-dependent mode of lithium efflux in the erythrocytes of pigs. Although mediation of SDLE in several mammalian species, including humans, is a function of a specific lithium-sodium countertransport system, we have not fully characterized the kinetic properties of lithium transport in pig erythrocytes and cannot assess the relationship of lithium-sodium countertransport to SDLE.

Lithium transport in red cells shows important species differences between swine and humans. First, although as in humans the leak component of lithium efflux (i.e., efflux into sodium-free media in the presence of ouabain) was similar whether magnesium, choline, or potassium was the predominant external cation, the absolute rate of lithium efflux was some fourfold to eightfold more rapid in pigs. The cotransport inhibitor bumetanide did not suppress lithium efflux into magnesium chloride, which suggests that coupled potassium-lithium-chloride efflux does not contribute to net lithium efflux under the conditions we employed. At present, the differences in lithium efflux into magnesium chloride are most compatible with differences in membrane permeability. A second characteristic of lithium efflux from pig erythrocytes that differed from that in humans was the source of interindividual variability. Variation in lithium efflux rates from human red cells results predominantly from differences in the maximum rate (V_{max}) of the sodium-dependent lithium-sodium countertransport system; lithium efflux through the leak pathway is relatively constant. In untreated pigs, the variability in the apparent leak pathway was similar to that for SDLE. Finally, the countertransport inhibitor phloretin has been shown to suppress lithium efflux from lithium-loaded human red cells to leak rates, but in our study it only partially inhibited lithium efflux from pig cells and then only at high concentrations. Species differences in the sensitivity of lithium fluxes to inhibition by phloretin have been reported by others for sheep, rabbit, and bovine red cells.

The results of these studies confirm and extend the previous observations of Guthe et al. on changes in...
red blood cell cation composition during the development of DOCA hypertension in pigs: compared with controls, in DOCA-treated pigs, cell sodium and water content rose while cell potassium content did not. The major new finding presented here is that of a progressive increase in SDLE concomitant with a decrease in lithium efflux into magnesium chloride.

The decrease in what is usually referred to as the leak component of lithium efflux, that is, ouabain-insensitive net transport into a medium free of both sodium and potassium, is seemingly at odds with previous observations of increased cell membrane permeability to sodium, potassium, lithium, and chloride in vascular tissues of rat models of DOCA-salt hypertension.18-21 However, changes in red blood cell cation transports in response to mineralocorticoid treatment have not been as extensively studied. Duhm et al.22 described enhanced sodium and rubidium diffusional leaks in deoxycorticosterone enanthate NaCl hypertensive rat erythrocytes but did not assess lithium movements. Noting that deoxycorticosterone enanthate treatment of rats without dietary sodium loading caused a significant increase in sodium but not in rubidium leak, the authors concluded that leak pathways for monovalent cations may differ.22 A more thorough examination of the changes in the kinetics of lithium transport induced by DOCA treatment in pig red cells and an examination of the relation of changes detected in red cells to cation metabolism in vascular tissues will be necessary to relate our observations to those in other species.

Since external magnesium has been shown to inhibit passive cation fluxes in human red cells,23 it should be emphasized that the differences in lithium fluxes we observed during our DOCA-salt experiments are not the result of inhibition by external magnesium. Although low concentrations of external magnesium were present in all our efflux media because we employed a magnesium chloride washing solution after lithium loading, any inhibitory effect of magnesium chloride was apparently maximal in all efflux media under the conditions employed, as there was no significant suppression of lithium efflux into MS (75 mM MgCl₂) compared with efflux into choline chloride solution or potassium chloride solution, in which residual external magnesium concentration was estimated to be about 5 mM.

The relationship of increases in SDLE induced by DOCA hypertension in the pig to the elevated countertransport activity associated with human essential hypertension is unknown. Importantly, in contradistinction to the changes observed in the pig, increased countertransport in human hypertension is due to increased sodium-stimulated efflux.1 Small differences in leak rates into magnesium chloride have been found in human hypertension by some,2 although not all,1,7 investigators, but clearly the major source of human interindividual heterogeneity is in sodium-stimulated efflux. Also, in vivo changes in lithium-sodium countertransport induced by pregnancy26 and dialysis27 and an in vitro effect of incubation22 are apparently mediated primarily by changes in sodium-stimulated efflux. Human phenotypic interindividual heterogeneity in countertransport has been assumed to reflect genetic polymorphism, but clearly the short-term changes induced in SDLE in pigs are more suggestive of modulation of the activity of an in situ transport function. Use of this model to study the factors capable of regulating lithium transport may lead to a better understanding of the basis of human countertransport heterogeneity.

In summary, we have demonstrated that DOCA treatment of pigs ingesting a diet of 200 mEq of sodium per day results in increases in red cell sodium and water content and SDLE while lithium efflux into an isotonic magnesium chloride–sucrose medium decreases. Total lithium efflux (sodium-stimulated plus diffusional leak) remains constant.

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