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Cellular Lithium and Transepithelial Transport Across Toad Urinary Bladder

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Summary. Toad urinary bladders were exposed on either their mucosal or serosal surfaces, or on both surfaces, to medium in which sodium was replaced completely by lithium. With mucosal lithium Ringer's, serosal sodium Ringer's, short-circuit current (SCC) declined by about 50 percent over the first 60 min and was then maintained over a further 180 min. Cellular lithium content was comparable to the sodium transport pool. With lithium Ringer's serosa, SCC was abolished over 60 to 120 min whether the mucosal cation was sodium or lithium. Measurements of cellular ionic composition revealed that the epithelial cells gained lithium from both the mucosal and serosal media. With lithium Ringer's mucosa and serosa, cells lost potassium and gained lithium and a little chloride and water, but these changes in cellular ions could not account for the current flow across the tissue under these conditions, which must, therefore, have been carried by a transepithelial movement of lithium itself. The inhibition by serosal lithium of SCC was overcome by exposure of the mucosal surface of the bladders to amphotericin B. Thus it reflected, predominantly, an inhibition of lithium entry to the cells across the apical membrane. It is suggested that this inhibition is a consequence of cellular lithium accumulation.

Key Words lithium · toad bladder · epithelial transport · cellular composition

Introduction

Many epithelia have, as one of their important physiological functions, the capacity to transport sodium by a metabolically dependent mechanism. In some epithelia, notably 'tight' epithelia such as amphibian skin (Galeotti, 1904; Zerahn, 1955) and urinary bladder (Herrera, Egea & Herrera, 1971), and turtle colon (Sarracino & Dawson, 1979), lithium, unlike all other ions, can substitute for sodium and be transported across the tissue from mucosa to serosa. Inasmuch as this transport is inhibited by amiloride (Herrera et al., 1971; Herrera, 1972; Candia & Chiarandini, 1973; Morel & Leblanc, 1975; Reinach, Candia & Siegel, 1975; Nagel, 1977; Sarracino & Dawson, 1979) and by ouabain (Herrera et al., 1971; Herrera, 1972; Candia & Chiarandini, 1973; Sarracino & Dawson, 1979), lithium appears to follow the same pathway as sodium in its passage from the outer (or mucosal) to the inner (or serosal) surface of these epithelia.

Though the effects of medium lithium on electrical parameters of epithelia have been documented, and the consequences of exposure to amiloride and to ouabain described, there is but little information on the composition of transporting epithelial cells exposed to lithium in the bathing media (Hansen & Zerahn, 1964; Herrera et al., 1971; Leblanc, 1972; Morel & Leblanc, 1975; Dolman, Edmonds & Salas-Coll, 1976). Such information is required to help resolve some of the outstanding questions related to transepithelial lithium transport.

We have chosen to examine the transepithelial electrical characteristics and epithelial cell composition of toad urinary bladders bathed on one or both surfaces by medium in which lithium replaced sodium completely to address the following questions. Is lithium actively transported across the epithelium or does its transepithelial movement reflect lithium entry to the cells from the mucosal medium with the current carried across the basolateral cellular membranes by potassium and/or chloride movements as has been suggested (Herrera et al., 1971), or by sodium-lithium countertransport as described for lithium extrusion from erythrocytes (Haas, Schooler & Tosteson, 1975; Duhm, Eisenried, Becker & Greil, 1976; Sarkadi, Alifimoff, Gunn & Tosteson, 1978; Duhm & Becker, 1979; Ehrlich & Diamond, 1979), and nerve cell cultures (Szentistványi et al., 1980)? How does the cellular lithium content of mucosal origin compare with the cellular sodium of mucosal origin – the so-called cellular transport pool (Macknight, Civan & Leaf, 1975a)? Does lithium enter the cells from the serosal medium? Why does serosal medium

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lithium inhibit transepithelial lithium and sodium transport? A preliminary report of a part of this study has been presented elsewhere (Macknight & Hughes, 1981).

Materials and Methods

The sodium Ringer's solution contained (mM): 117 Na^+, 4.0 K^+, 1.0 Ca^{2+}, 1.0 Mg^{2+}, 119 Cl^-, 1.0 SO_4^{2-}, and 10 glucose, buffered at pH 7.8 by 2 mM HPO_4^{2-}. Sodium-free lithium Ringer's and choline Ringer's solutions were otherwise identical but contained either 117 mM lithium or choline chloride substituted for 117 mM sodium chloride.

Inulin-carboxyl-^{14}C and inulin-methoxy-^{3}H were obtained from New England Nuclear Corporation. Ouabain, EGTA, and dibutyryl cAMP were obtained from Sigma Chemical Corp., amphotericin B from E.R. Squibb & Sons Pty Ltd., and vasopressin from Park Davis & Co. Amiloride was the gift of Merck, Sharp and Dohme.

Toads of the species *Bufo marinus*, obtained from the Dominican Republic (National Reagents, Bridgeport, Conn.) were doubly pithed and their hearts immediately perfused with sodium Ringer's to deblood the bladders.

Paired hemibladders were mounted in chambers of area 8.04 cm^2. Initially, both mucosal and serosal surfaces were bathed with sodium Ringer's and the open-circuit potential difference (PD) recorded. They were then continuously short-circuited except for brief interruptions to determine PD. Air was bubbled through both mucosal and serosal solutions throughout the experiments.

Once the short-circuit current (SCC) had stabilized, the chambers were drained and refilled with the appropriate solutions as indicated. Whenever it was desired to bathe the hemibladders with sodium-free solutions the appropriate chambers were drained, then filled and drained five times using fresh sodium-free Ringer's before filling. Chambers were also drained, washed and refilled periodically during the course of the experiments as indicated in the graphs. This procedure allowed washout of sodium from the tissue and prevented any appreciable accumulation of sodium in the final solution during the course of the experiment. Both the mucosal and serosal solutions contained radioactive inulin for at least 60 min before the end of each experiment. Since there is no measurable flux of inulin across the bladder, the use of ^{3}H-inulin in one solution and ^{14}C-inulin in the other allowed separate correction of tissue composition for any mucosal and serosal extracellular fluid in the sample.

With solutions of different composition bathing the two surfaces of the epithelium, chemical potential gradients exist for lithium and for sodium movements across the epithelia, diffusion potentials may arise, and short-circuit current may no longer be equated only with active ion transport from mucosa to serosa. However, with lithium Ringer's substituted for either mucosal or serosal sodium Ringer's alone, open circuit PD (and, therefore, SCC) was abolished by amiloride (Fig. 5) and by ouabain (Fig. 6). Therefore, it can be concluded that sodium and lithium diffusion potentials must be of similar magnitude and, since the diffusion gradients are the same, that the passive pathway for these ions across this tissue (the paracellular pathway) has a similar selectivity for both ions. Thus, even under these experimental conditions, SCC provided an accurate estimate of mucosa-to-serosa sodium or lithium movements through the active (cellular) transport pathway.

At the conclusion of each experiment, the chambers were drained, the portion of the hemibladder exposed in the chambers removed, and blotted several times on Whatman filter paper no. 542 until no visible moisture was transferred to the paper. It was then placed mucosal surface up on a Pyrex® petri dish and the epithelial cells were removed by scraping with a glass slide. It has been shown that this procedure removes over 90% of the epithelial cells from the underlying tissue without contamination by other tissue elements (Macknight, DiBona, Leaf & Civan, 1971). The epithelial cell scrapings were then transferred to tarred Pyrex tubes.

Scrapings were weighed, dried to constant weight at 105° C in a hot air oven, cooled at room temperature for 40 min, and reweighed. The water content of the scraped tissue was taken to be equal to the loss of weight upon drying.

In all experiments, tissue was extracted for 7–10 days in 10 ml of 0.1 M nitric acid. Sodium, potassium and lithium were measured with an EEL flame photometer and chloride with a Cotlove titrator (Cotlove, Trantham & Bowman, 1958). The lithium readings obtained from the flame photometer were corrected for the small amount of potassium interference which was present.

Inulin-^{14}C and inulin-^{3}H were determined by adding 17 ml Triton X-100-toluene fluor to 2 ml tissue extract. The samples were then counted in a three channel Packard Tri-Carb Liquid Scintillation counter. Samples of medium, 0.1 ml, were diluted by the addition of 2 ml 0.1 M nitric acid and counted in the same way.

The tissue values of water and ions were corrected for contamination with extracellular fluid using the assumption that isotopically labeled inulin equilibrated in the extracellular space and that the ions in this space were at the same concentration as in the bulk of the medium. These derived values are referred to as the noninulin space or cellular contents.

The cellular water content is expressed as kg H_2O/kg dry weight. The derived ion contents are shown in mmoles/kg dry weight. Their concentrations in mmoles/kg cellular water can be calculated by dividing the ion content by the cellular water content.

Transepithelial electrical resistance (R_t) was either calculated from the measured open circuit PD and SCC, or was determined by periodically imposing a 10-mV potential difference across the tissue for 30 sec and measuring the resultant deflection of transepithelial current. This technique was used particularly under conditions where open circuit PD and SCC had fallen to very low values. The transepithelial resistance is expressed in ohms cm^2.

Values presented in text and Tables represent the mean ± SEM where appropriate, of the number of observations shown in parentheses. Means of paired analyses are presented with their difference and its SEM. Significance of differences between means have been evaluated using Student's *t* test.

Results

Lithium Ringer's Mucosa and Serosa

With lithium substituted for sodium in both the mucosal and serosal media, transepithelial PD and SCC were depressed. A typical result is illustrated (Fig. 1), and the averaged data are presented in Table 1. Both PD and SCC declined steadily after the substitution, and, on average, were virtually abolished between 60 and 120 min after exposure to the lithium. Since both parameters decreased together, estimated tissue resistance was not altered significantly in the absence of medium sodi-

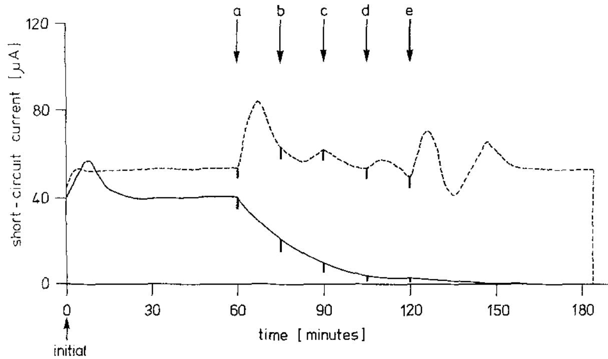

Fig. 1. Effect on SCC of substitution of lithium Ringer's for mucosal and serosal sodium Ringer's. Prior to *a*, both hemibladders were incubated with sodium Ringer's mucosa and serosa. This is true also for all subsequent Figures. At *a* chambers were drained and refilled five times with either fresh sodium Ringer's (-----) or lithium Ringer's (——). These washings were repeated at *b, c, d* and *e*. Following washing with sodium Ringer's, SCC was often stimulated. This behavior is typical of the effect of washing in this way and will be noticed in other Figures in this series. Washing with lithium Ringer's did not, as a rule, provoke such transient stimulation. Periodically, a 10-mV pulse was placed across the tissue and the resulting deflection in current recorded. These deflections have been omitted from the redrawn tracings for clarity

um. The slow steady decline in transport with lithium Ringer's mucosa and serosa can be contrasted with the dramatic effects of replacing medium sodium by choline (Fig. 2), and of amiloride (Fig. 5), both of which abolished transepithelial transport over a few minutes. In a separate series of five experiments, the actual net ion flux across the tissue before the SCC fell to zero following the substitution for sodium of lithium Ringer's mucosa and serosa, was measured by planimetry from the recorder tracings. It averaged 364±23 mmol/kg dry wt of epithelial cell scrapings. This can be compared with the average rate of transepithelial sodium transport reported previously in a large series of experiments ($n=45$), of 2,164 mmol/kg dry wt per hr (Macknight, Civan & Leaf, 1975*b*). Thus an appreciable current flow does occur during the decline in transport induced by lithium.

The replacement of medium sodium by lithium resulted in marked changes in noninulin space composition (Table 2), which were largely completed within 60 min. Compared to hemibladders exposed to sodium Ringer's, there were significant decreases in noninulin space sodium and potassium contents. The lithium gained more than offset these losses and, after 60 min, both chloride and water contents had risen significantly. Of the 325 mmol/kg dry wt of lithium gained, 147 mmol replaced potassium, and 122 mmol replaced sodium. The remaining 56 mmol/kg dry wt were ac-

Table 1. Effects on electrical parameters of substitution of lithium Ringer's for mucosal (*m*) and serosal (*s*) sodium Ringer's[a]

Medium	PD (mV)	SCC (μA)	R_T (ohm cm^2)
a) 60-min incubation with Li Ringer's *m&s*:			
1) Before exposure to Li Ringer's–			
Na Ringer's (17)	53± 6	90±13	5500± 700
Na Ringer's (17)	51± 6	96±20	5700± 800
Δ	3± 5	6±11	200± 800
P	>0.50	>0.50	>0.80
After 60-min exposure to Li Ringer's *m* & *s*–			
Li Ringer's (17)	3± 1	6± 2	4700± 600
Na Ringer's (17)	45± 6	104±16	3700± 300
Δ	42± 5	98±17	1000± 600
P	<0.001	<0.001	>0.10
b) 120-min incubation with Li Ringer's *m* & *s*:			
1) Before exposure to Li Ringer's–			
Na Ringer's (7)	45±10	70±16	5200± 900
Na Ringer's (7)	47±11	70± 9	5200± 900
Δ	2± 9	1± 9	0±1000
P	>0.80	>0.95	>0.98
2) After 120-min exposure to Li Ringer's *m* & *s*–			
Li Ringer's (7)	0± 1	−2± 2	3900± 700
Na Ringer's (7)	55±13	94±15	4500± 400
Δ	55±13	96±14	600± 600
P	<0.005	<0.001	>0.40

[a] Paired hemibladders were initially incubated with sodium Ringer's bathing both surfaces. Once their SCC had stabilized, chambers were drained. (The identical procedure was used in experiments, the results of which are shown in all subsequent Tables.) One hemibladder was then incubated with lithium Ringer's *m* & *s* while the paired hemibladder was incubated with fresh sodium Ringer's for either 60 or 120 min before being taken for analysis of epithelial composition. Both hemibladders were subjected to washing and replacement of their media as described in Materials and Methods and illustrated in Fig. 1. Note that there were no significant differences in the electrical parameters between the paired hemibladders when incubated initially with sodium Ringer's. In all subsequent experiments this was also true. For this reason, the initial values are not presented in the Tables which follow. However, when neither hemibladder was incubated subsequently with sodium Ringer's bathing both surfaces, averaged values for the initial incubation in sodium Ringer's from all hemibladders are given in the Table legend.

companied by an uptake of 67 mmol/kg dry wt of chloride and 0.47 kg/kg dry wt of water, as would be required to maintain osmotic equilibrium between cells and the bathing media. Similar changes were also found in hemibladders exposed to lithium Ringer's for 120 min for, of the 406 mmol/kg dry wt of lithium accumulated, 170 mmol replaced potassium, 178 mmol replaced sodium, and 58 mmol was associated with an apparent gain of 74 mmol chloride and 0.44 kg/kg dry wt of water. Thus, within the experimental error, there was an

uptake of an isosmotic solution of lithium chloride as well as a loss of potassium and of sodium in exchange for medium lithium.

After both 60- and 120-min incubation in sodium-free mucosal and serosal lithium Ringer's, there remained a small amount of sodium which had not exchanged with lithium. Similarly, in sodium-free choline Ringer's there is always some small quantity of sodium which remains even after several hours incubation (Hughes & Macknight, *unpublished observations*). Previous work has revealed that within experimental error all noninulin space sodium exchanges with ^{24}Na in sodium Ringer's (Macknight, Civan & Leaf, 1975*a*), and the reasons for the failure of a small quantity of sodium to exchange with these other monovalent cations remain unclear.

The changes in ionic composition which had occurred by the time the SCC had fallen to zero were determined for the five hemibladders in which total net current flow after substitution of lithium for mucosal and serosal sodium was calculated. Compared with their paired controls incubated throughout in sodium Ringer's, the tissues incubated with lithium lost 202±14 mmol/kg dry wt of sodium, and 201±14 mmol/kg dry wt of potassium, while gaining 445±33 mmol/kg dry wt of lithium and 56±25 mmol/kg dry wt of chloride.

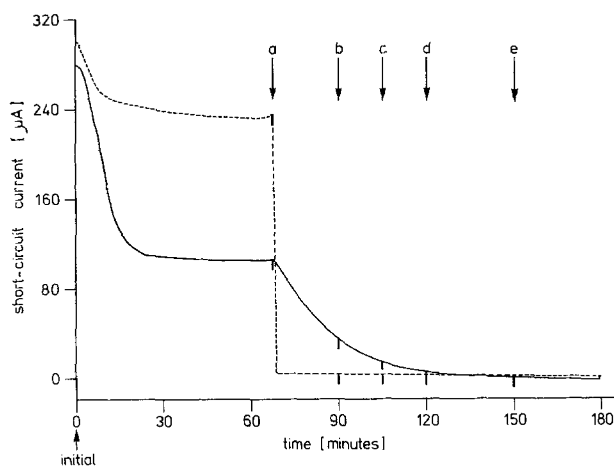

Fig. 2. Effect on SCC of substitution of lithium Ringer's or choline Ringer's for mucosal and serosal sodium Ringer's. At *a* chambers were drained and refilled five times with either choline Ringer's (-----) or lithium Ringer's (——). These washings were repeated at *b*, *c*, *d* and *e*

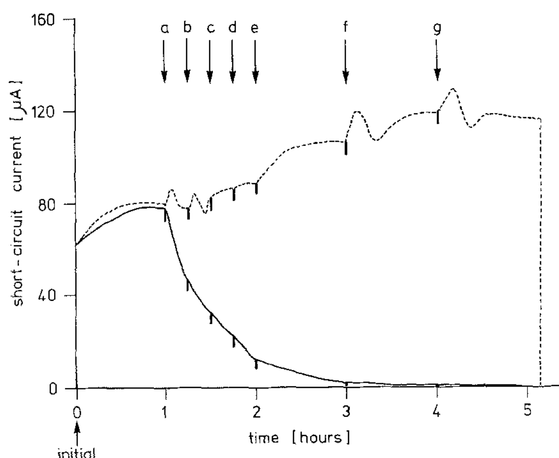

Fig. 3. Effect on SCC of substitution of lithium Ringer's for serosal sodium Ringer's. At *a* chambers were drained and refilled five times with either sodium Ringer's mucosa and serosa (-----) or sodium Ringer's mucosa, lithium Ringer's serosa (——). These washings were repeated at *b*, *c*, *d*, *e*, *f* and *g*

Table 2. Effects on noninulin space epithelial composition of exposure to lithium Ringer's bathing both surfaces[a]

Medium	H_2O (kg/kg dry wt)	Non-inulin space			
		Na	K (mmol/kg dry wt)	Li	Cl
a) After exposure for 60 min:					
Na Ringer's (17)	2.35±0.16	153±12	378±12		222±13
Li Ringer's (17)	2.83±0.17	32± 4	231±11	325±22	290±20
Δ	0.47±0.16	122±14	147±16	325	67±21
P	<0.01	<0.001	<0.001		<0.005
b) After exposure for 120 min:					
Na Ringer's (7)	2.76±0.24	198±12	401±16		268±12
Li Ringer's (7)	3.20±0.27	20± 3	230±19	406±31	342±36
Δ	0.44±0.30	178±12	170±12	406	74±44
P	>0.10	<0.001	<0.001		>0.10

[a] Paired hemibladders were incubated as described in Table 1 which presents the averaged electrical parameters for these tissues.

Sodium Ringer's Mucosa, Lithium Ringer's Serosa

Substitution of lithium for sodium in the serosal medium alone, also resulted in a marked inhibition of SCC (Fig. 3, Table 3*a*) and an appreciable fall in PD. Again, since both parameters fell in parallel, there was no significant change in calculated tissue resistance. Direct comparison of the rate of inhibition of net mucosal-to-serosal cation movement with lithium Ringer's serosa revealed no significant difference whether sodium or lithium was the dominant mucosal cation (Table 3*b*).

After 120 min, tissues exposed to serosal lithium Ringer's had gained 181 mmol/kg dry wt of lithium from that medium, partly in exchange for potassium and partly for sodium (Table 4). Water and chloride contents had not altered significantly. With sodium in the mucosal medium, the noninulin space sodium content was 57 mmol/kg dry wt, a value appreciably greater than the 120-min value of 20 mmol/kg dry wt in tissues exposed to lithium Ringer's mucosa and serosa (Table 2).

Table 3. Effects on electrical parameters of substitution of lithium Ringer's for sodium Ringer's[a]

	PD (mV)	SCC (μA)	R_t (ohm cm^2)
a) Lithium Ringer's serosa compared with sodium Ringer's serosa, with sodium Ringer's mucosa:			
Serosal medium			
1) After 60-min incubation with Li Ringer's serosa–			
Li Ringer's (18)	12±2	18±2	5300±500
Na Ringer's (18)	29±4	42±6	5900±400
Δ	16±4	25±6	600±500
P	<0.001	<0.001	>0.20
2) After 120-min incubation with Li Ringer's serosa–			
Li Ringer's (18)	6±1	9±1	5000±500
Na Ringer's (18)	33±4	53±8	5800±400
Δ	27±4	44±8	700±400
P	<0.001	<0.001	>0.05
b) Lithium Ringer's serosa, either sodium or lithium Ringer's mucosa:			
Media			
1) After 60-min incubation with lithium Ringer's–			
Na Ringers's *m*			
Li Ringer's *s* (8)	5±1	8±3	5800±600
Li Ringer's *m*			
and *s* (8)	5±1	8±2	5500±500
Δ	0±1	0±1	300±500
P	>0.80	>0.80	>0.50
2) After 120-min incubation with lithium Ringer's–			
Na Ringer's *m*			
Li Ringer's *s* (8)	2±1	3±2	5700±500
Li Ringer's *m*			
and *s* (8)	2±1	2±1	5200±300
Δ	0±1	1±2	500±500
P	>0.50	>0.80	>0.30

[a] In part *b*) initial values with sodium Ringer's *m* and *s* averaged 23±5 mV, 30±8 μA and 6700±700 ohm cm^2.

Lithium Ringer's Mucosa Sodium Ringer's Serosa

Substitution of lithium for sodium in the mucosal medium alone, caused a depression of both PD and SCC (Fig. 4, Tables 5 & 6). Howevever, in

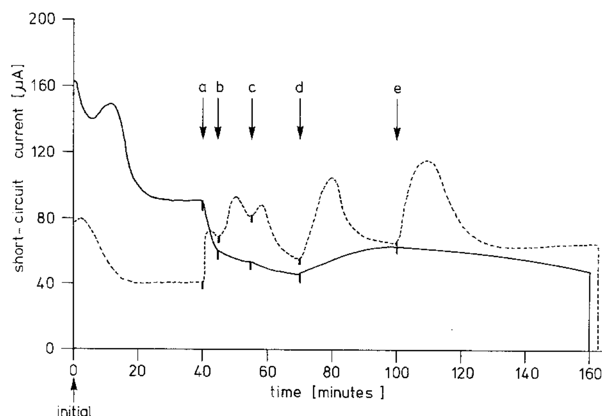

Fig. 4. Effect on SCC of substitution of lithium Ringer's for mucosal sodium Ringer's. At *a* chambers were drained and refilled five times with either sodium Ringer's mucosa and serosa (-----) or lithium Ringer's mucosa, sodium Ringer's serosa (———). These washings were repeated at *b, c, d* and *e*

Table 4. Effects on epithelial noninulin space composition of exposure for 120 min to lithium Ringer's bathing the serosal surface with sodium Ringer's bathing the mucosal surface[a]

Serosal medium		H_2O (kg/kg dry wt)	Non-inulin space: Na	K	Li	Cl
			(mmol/kg dry wt)			
Na Ringer's (18)		2.47±0.14	182±18	361±11		259±18
Li Ringer's (18)		2.44±0.18	57±10	260±13	181±17	247±18
	Δ	0.03±0.15	125±23	101±11	181	12±21
	P	>0.80	<0.001	<0.001		>0.50

[a] Paired hemibladders were incubated as described in Table 3*a* which presents the averaged electrical parameters for these tissues.

contrast to the inhibitory effects of serosal lithium, mucosal lithium resulted in a decrease of only about 50 percent in SCC, and transport continued at this reduced level for at least 240 min (Table 5).

With mucosal lithium alone (Table 6), the changes in tissue composition over 60 min were much less than those associated with serosal lithium (Tables 2 & 4). Tissue gained 66 mmol/kg dry wt of lithium and lost 47 mmol of sodium. The decrease of 20 mmol/kg of potassium was not statistically significant, and neither water nor chloride contents changed significantly. In four additional experiments lithium content after 240-min incubation was 67±11 mmol/kg dry wt with comparable changes in tissue sodium and potassium to those measured in the much larger series of 60-min experiments.

With sodium Ringer's bathing the mucosal surface, about 15 mmol/kg dry wt of sodium are removed from the noninulin space by washing the mucosal surface with sodium-free choline Ringer's containing 10^{-4} M amiloride (Macknight et al., 1975*a*). Similarly, with tissues incubated in lithium Ringer's mucosa sodium Ringer's serosa, washing the mucosal surface with choline Ringer's containing 10^{-4} M amiloride removed 15±4 mmol lithium ($P<0.005$), reducing the noninulin space lithium in this series of 17 experiments from 77±6 to 62±6 mmol/kg dry wt.

Table 5. Effects on electrical parameters of substitution of lithium Ringer's for mucosal sodium Ringer's[a]

Mucosal medium	PD (mV)	SCC (μA)	R_t (ohm cm^2)
a) After 120-min incubation with Li Ringer's mucosa:			
Li Ringer's (4)	15±2	19± 6	7400±1400
Na Ringer's (4)	33±7	52± 8	5200± 900
Δ	18±7	33± 5	2200±1500
P	>0.05	<0.01	>0.20
b) After 240-min incubation with Li Ringer's mucosa:			
Li Ringer's (4)	16±1	28± 8	5400±1200
Na Ringer's (4)	36±6	68±12	4900± 600
Δ	21±6	40± 5	500±1200
P	<0.05	<0.005	>0.70

[a] After initial incubation with sodium Ringer's, one hemibladder was then incubated with lithium Ringer's mucosa, sodium Ringer's serosa while the paired hemibladder was incubated with fresh sodium Ringer's. All tissues were incubated for 240 min before being taken for analysis of epithelial composition. The values at 60 and 180 min were similar to those at 120 and 240 min.

Effects of Amiloride

As illustrated in Fig. 5*A*, amiloride, 10^{-4}M, which inhibits sodium entry to the cells across the apical membrane (Bentley, 1968), completely abolished PD (1±1 mV) and SCC (1±2 μA) with lithium Ringer's mucosa, sodium Ringer's serosa ($n=15$). Tissue resistance was significantly increased by amiloride from 3,300±300 ohm cm^2 to 4,800±600 ohm cm^2 (*Δ*1,500±700, $P<0.05$). As-

Table 6. Effects on noninulin space epithelial composition, and on associated electrical parameters, of exposure to lithium Ringer's mucosa, sodium Ringer's serosa for 60 min[a]

Mucosal medium	H_2O (kg/kg dry wt)	Noninulin space			
		Na	K	Li	Cl
		(mmol/kg dry wt)			
Li Ringer's (17)	2.39±0.11	130±10	360±9	66±6	225±12
Na Ringer's (17)	2.40±0.14	177±10	379±12		235±10
Δ	0.01±0.14	47±9	20±12	66	10±13
P	>0.98	<0.001	>0.10		>0.40

Electrical parameters

	PD (mV)	SCC (μA)	R_t (ohm cm^2)
After 60-min incubation:			
Li Ringer's (17)	16±2	45±9	3500±300
Na Ringer's (17)	45±7	106±19	3700±300
Δ	29±7	62±18	200±300
P	<0.005	<0.005	>0.40

[a] After initial incubation with sodium Ringer's, one hemibladder was then incubated for 60 min with lithium Ringer's mucosa, sodium Ringer's serosa while the paired hemibladder was incubated with fresh sodium Ringer's.

sociated with this inhibition of transport, tissues gained less lithium and lost less potassium (Table 7*a*). In a separate series of six experiments, washing the mucosal surface with choline Ringer's containing 10^{-4} M amiloride removed 22 ± 7 mmol/kg dry wt ($P < 0.025$) decreasing noninulin space lithium from 33 ± 6 to 11 ± 2 mmol/kg dry wt.

With lithium Ringer's mucosa and serosa, amiloride, 10^{-4} M, added to mucosal medium after 10 min, before transport had been substantially inhibited by serosal lithium, also immmediately abolished SCC (Fig. 5*B*). However, the values of PD and SCC reached after 80-min exposure to lithium were very similar whether or not amiloride was present (PD's 0 ± 1, 1 ± 1 mV; SCC's 1 ± 3, 2 ± 2 µA). Tissue resistance was increased significantly in the presence of amiloride (6,300 ±

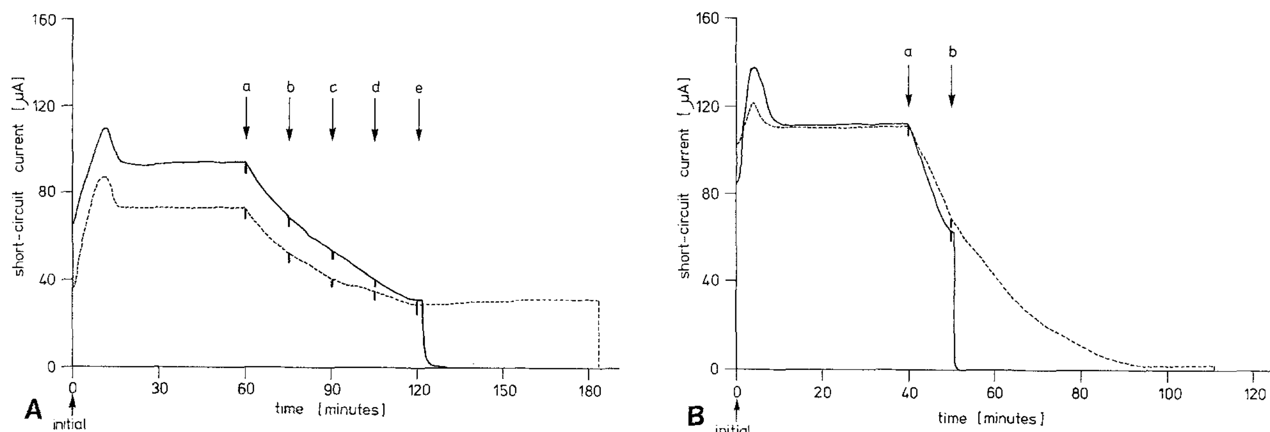

Fig. 5. Effect on SCC of amiloride (10^{-4} M). *A*) Lithium Ringer's mucosa, sodium Ringer's serosa. *B*) Lithium Ringer's mucosa and serosa. At *a* chambers were drained and refilled five times with lithium or sodium Ringer's as appropriate. In *A*) these washings were repeated at *b*, *c* and *d*. At *e* (Fig. 5*A*) and at *b* (Fig. 5*B*) amiloride was added to the mucosal medium bathing one of the hemibladders. (——)

Table 7. Effects of amiloride, 10^{-4}M, on noninulin space epithelial composition

Mucosal medium		H_2O (kg/kg dry wt)	Noninulin space Na	K	Li	Cl
			(mmol/kg dry wt)			
a) After exposure to lithium Ringer's as the mucosal medium:[a]						
Li Ringer's (15)		2.87±0.12	150±13	400± 7	84± 7	246±12
amiloride (15)		2.85±0.14	169±14	434±14	42± 7	270±15
	Δ	0.02±0.14	19±20	34±13	42± 8	23±18
	P	>0.80	>0.30	<0.025	<0.001	>0.20
b) After exposure to lithium Ringer's mucosa and serosa:[b]						
Li Ringer's (8)		2.76±0.28	37± 7	215±11	213±19	250±14
+amiloride (8)		2.28±0.17	42± 6	266±14	153±14	184±17
	Δ	0.48±0.32	5± 9	51±16	60±22	67±24
	P	>0.10	>0.50	<0.02	<0.05	<0.05

[a] After initial incubation with sodium Ringer's, both hemibladders were then incubated with lithium Ringer's mucosa, sodium Ringer's serosa for 60 min before amiloride, 10^{-4}M final concentration, was added to the mucosal medium bathing one hemibladder. Both hemibladders were then incubated for a further 60 min before being taken for analysis of epithelial composition. The SCC from a typical experiment is illustrated in Fig. 5*A*.

[b] After initial incubation with sodium Ringer's, both hemibladders were then incubated with lithium Ringer's mucosa and serosa for 10 min before amiloride, final concentration 10^{-4}M, was added to the mucosal medium bathing one hemibladder. Both hemibladders were then incubated a further 60 min before being taken for analysis of epithelial composition. The SCC from a typical experiment is illustrated in Fig. 5*B*.

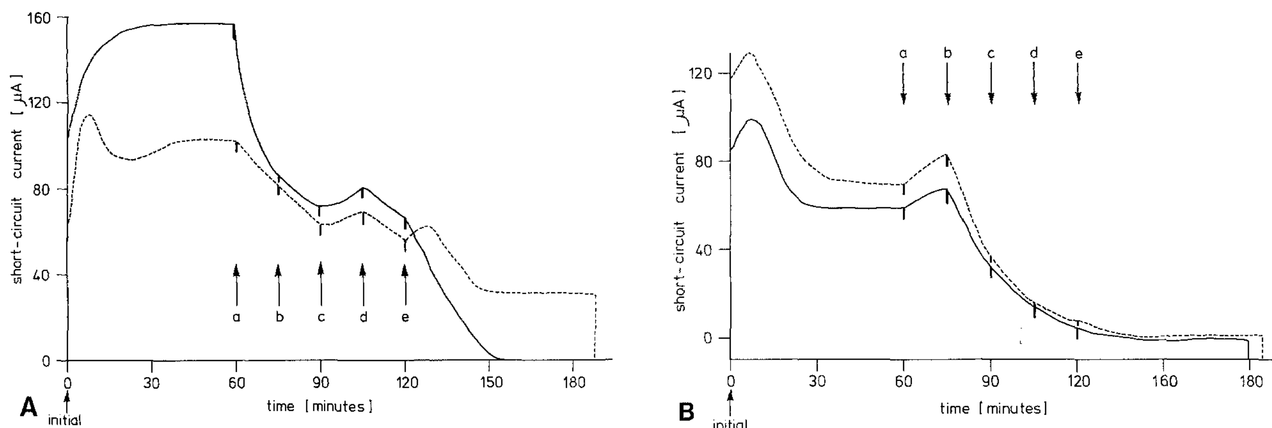

Fig. 6. Effect on SCC of ouabain (10^{-2} M). *A*) Lithium Ringer's mucosa, sodium Ringer's serosa. *B*) Lithium Ringer's mucosa and serosa. At *a* chambers were drained and refilled five times with lithium or sodium Ringer's as appropriate. These washings were repeated at *b, c* and *d*. At *e* serosal surfaces were exposed to ouabain (——) or to fresh media without the glycoside (-----)

500 ohm cm^2), compared to 4,700±200 ohm cm^2 without amiloride, $n=8$, Δ1,600±500 ($P<0.02$).

Tissues exposed to amiloride contained significantly less lithium, and more potassium than the paired tissues (Table 7*b*). (They may also have contained less chloride and water, but the scatter in these values, together with the fact that there is no difference in the sums of potassium + lithium contents under the two conditions, makes this doubtful.) The decrease in lithium content of 60 mmol/kg dry wt associated with amiloride under these conditions is comparable to the 66 mmol/kg dry wt of lithium gained from the mucosal medium alone with lithium Ringer's mucosa, sodium Ringer's serosa (Table 7a), showing that most of the lithium gained by tissues incubated with lithium Ringer's mucosa and serosa, is derived from the serosal medium. This interpretation is supported by a comparison of lithium contents of tissues incubated in sodium Ringer's mucosa, lithium Ringer's serosa (181 mmol/kg dry wt, Table 4) with lithium contents of tissues incubated in lithium Ringer's mucosa, sodium Ringer's serosa (66 mmol/kg dry wt, Table 6).

Effects of Ouabain

Exposure of hemibladders bathed by lithium Ringer's mucosa, sodium Ringer's serosa to ouabain, 10^{-2} M, abolished SCC (Fig. 6*A*) and PD, as described by others in toad urinary bladder (Herrera et al., 1971; Herrera, 1972). Analysis of tissue composition (Table 8*a*) revealed that tissues exposed to ouabain contained significantly less potassium and less lithium, but more sodium (of serosal origin). Water and chloride contents were unaffected.

With lithium Ringer's mucosa and serosa, ouabain 10^{-2} M, added to the serosal medium after 60 min lithium Ringer's, had no further inhibitory effect on the electrical parameters, for in both groups SCC (Fig. 6*b*) and PD were abolished after 120 min (PD's -1 ± 1, -1 ± 1; SCC's -2 ± 1, -1 ± 2, $n=7$). Nor was tissue composition affected by ouabain (Table 8*b*). Inasmuch as SCC was markedly depressed before the addition of ouabain, ouabain may not actually have bound to pump sites, for there is evidence from tissue culture studies (Mills et al., 1981) that ouabain binds only to functioning sites. However, addition of ouabain to the serosal lithium medium at the beginning of the exposure to lithium did not appear to cause a faster inhibition of SCC than did lithium alone.

Effects of Vasopressin and of Amphotericin B

The virtually complete inhibition of transepithelial ion transport which followed incubation of hemibladders in serosal lithium Ringer's could have resulted from either an inhibition of cation (sodium or lithium) entry to the cells from the mucosal medium or from an inhibition of cation extrusion from the cells to the serosal medium. In an attempt to distinguish between these two possibilities, the effects of two agents which normally promote transepithelial sodium transport by facilitating the entry of sodium to the cells from the mucosal medium were examined.

With lithium Ringer's mucosa and serosa, ex-

Table 8. Effects of ouabain, 10^{-2} M, on noninulin space epithelial composition

Serosal medium		H_2O (kg/kg dry wt)	Noninulin space			
			Na	K	Li	Cl
			(mmol/kg dry wt)			
a) After exposure to lithium Ringer's mucosa:[a]						
Na Ringer's (29)		2.55±0.12	174±12	326±14	59± 4	264± 8
+ouabain (29)		2.46±0.10	242±11	247± 8	29± 3	263± 7
	Δ	0.09±0.09	68±13	79±11	30± 5	1±11
	P	>0.30	<0.001	<0.001	<0.001	>0.99
b) After exposure to lithium Ringer's mucosa and serosa:[b]						
Li Ringer's (7)		2.70±0.19	44± 9	214±11	342±21	237±12
+ ouabain (7)		2.64±0.26	43± 7	199±10	361±19	260±14
	Δ	0.06±0.23	1± 8	15± 9	19±14	23±19
	P	>0.80	>0.90	>0.10	>0.20	>0.20

[a] After initial incubation with sodium Ringer's, both hemibladders were then incubated with lithium Ringer's mucosa, sodium Ringer's serosa for 60 min before the serosal surface of one hemibladder was exposed to sodium Ringer's containing 10^{-2}M ouabain. After a further 60 min both hemibladders were taken for analysis of epithelial composition. The SCC from a typical experiment is illustrated in Fig. 6*A*.

[b] After initial incubation with sodium Ringer's, both hemibladders were then incubated with lithium Ringer's mucosa and serosa for 60 min before the serosal surface of one hemibladder was exposed to lithium Ringer's containing 10^{-2}M ouabain. After a further 60 min both hemibladders were taken for analysis of epithelial composition. The SCC from a typical experiment is illustrated in Fig. 6*B*.

posure to vasopressin after 120 min in seven experiments resulted in a small, though significant absolute increase in both PD (from 1±0.2 mV to 3±0.5 mV, *Δ*2, $P<0.005$) and SCC (from 1±1 to 8±1 μA, *Δ*7, $P<0.001$). Earlier exposure to vasopressin (after 10 min in lithium Ringer's as transport was declining) caused a small immediate stimulus but did not prevent the subsequent decline (Fig. 7). There were no detectable changes in noninulin space composition after vasopressin under these experimental conditions. Exposure of tissues to dibutyryl cAMP (10^{-3}M) after 120-min incubation with lithium Ringer's mucosa and serosa likewise produced no appreciable stimulation of transepithelial lithium movement.

After incubation for 120 min with sodium Ringer's mucosa, lithium Ringer's serosa, exposure to vasopressin resulted in a marked stimulation both of PD and of SCC (Table 9*a*, Fig. 8), together with a significant decrease in tissue resistance (Table 9*a*). However, the SCC after vasopressin was still substantially less than the control value measured initially in sodium Ringer's. Though sodium did increase, there were no statistically significant changes in tissue composition after vasopressin under these experimental conditions. Thus, serosal lithium, itself, did not prevent some expression of the physiological response to vasopressin.

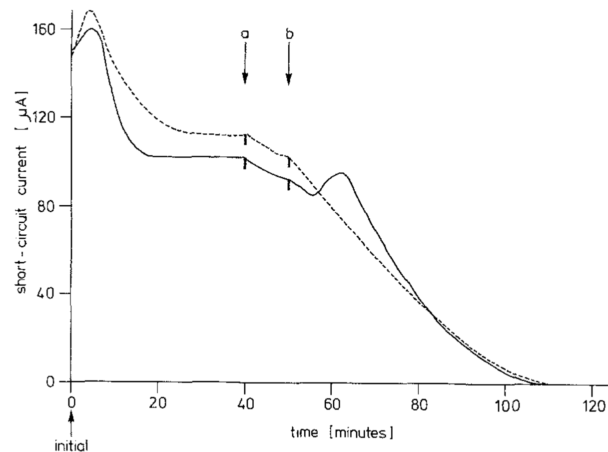

Fig. 7. Effect on SCC of vasopressin (100 mU/ml) after brief exposure to lithium Ringer's mucosa and serosa. At *a* chambers were drained and refilled five times with lithium Ringer's mucosa and serosa. These washings were repeated at *b* and vasopressin was added to the serosal medium bathing one hemibladder (——) to a final concentration of 100 mU/ml

After incubation for 120 min with lithium Ringer's mucosa, sodium Ringer's serosa, exposure to vasopressin also resulted in a marked stimulation both of PD and of SCC (Table 9*b*, Fig. 9), together with a significant decrease in tissue resistance (Table 9*b*). Here the SCC after vasopressin actually exceeded the initial control value in sodi-

Table 9. Effects on electrical parameters of vasopressin, 100 mU/ml[a]

	PD (mV)	SCC (μA)	R_t (ohm cm^2)
a) After exposure to sodium Ringer's mucosa, lithium Ringer's serosa:			
Serosal media			
Na Ringer's (14)	74±6	120±17	5000±700
After 120-min incubation with sodium Ringer's mucosa, lithium Ringer's serosa–			
Lithium Ringer's (14)	14±6	15± 3	5700±500
At peak of response to vasopressin–			
Lithium Ringer's +vasopressin (7)	36±1	85± 6	2800±600
Lithium Ringer's (7)	10±4	11± 9	6200±700
Δ	27±4	74±10	3400±600
P	<0.001	<0.001	<0.005
b) After exposure to lithium Ringer's mucosa, sodium Ringer's serosa:			
Mucosal medium			
Na Ringer's (14)	53±6	79±12	4800±900
After 120-min incubation with lithium Ringer's mucosa, sodium Ringer's serosa–			
Lithium (14)	11±2	28± 5	3100±400
At peak of response to vasopressin–			
Lithium Ringer's + Vasopressin (7)	21±2	102±15	1700±200
Lithium Ringer's (7)	10±3	27± 5	3000±400
Δ	11±3	74±17	1300±300
P	<0.01	<0.005	<0.005

[a] After initial incubation with sodium Ringer's, hemibladders were then incubated with lithium Ringer's serosa (*a*) or mucosa (*b*) for 120 min before vasopressin was added to the serosal medium bathing one hemibladder to give a final concentration of 100 mU/ml. Average values from all hemibladders are shown for the incubations preceding the addition of vasopressin, there being no significant differences in electrical parameters between the paired hemibladders.

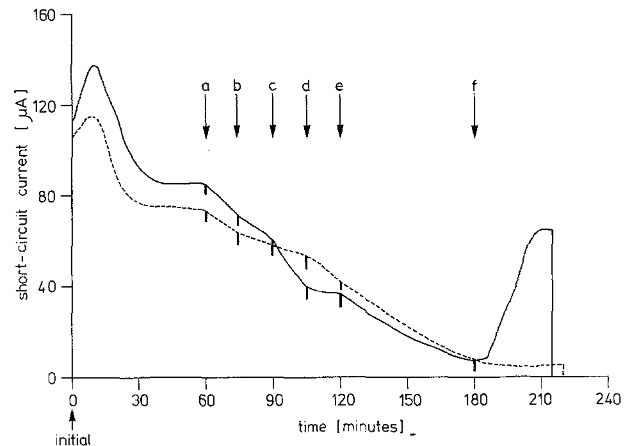

Fig. 8. Effect on SCC of vasopressin (100 mU/ml) with sodium Ringer's mucosa, lithium Ringer's serosa. At *a* chambers were drained and refilled five times with sodium Ringer's mucosa, lithium Ringer's serosa. These washings were repeated at *b*, *c*, *d* and *e*. At *f* vasopressin was added to the serosal medium bathing one hemibladder (———) to a final concentration of 100 mU/ml

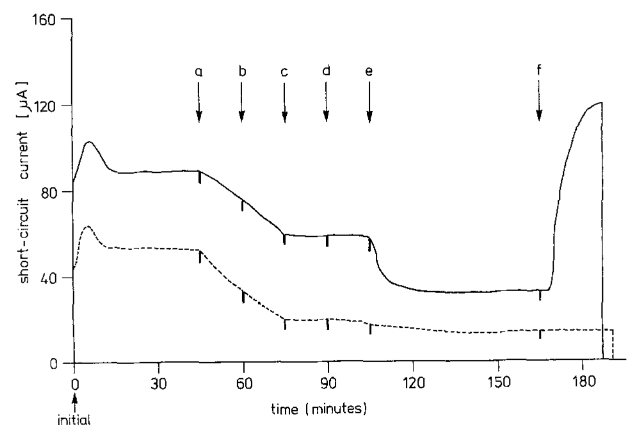

Fig. 9. Effect on SCC of vasopressin (100 mU/ml) with lithium Ringer's mucosa, sodium Ringer's serosa. At *a* chambers were drained and refilled five times with lithium Ringer's mucosa, sodium Ringer's serosa. These washings were repeated at *b*, *c*, *d* and *e*. At *f* vasopressin was added to the serosal medium bathing one hemibladder (———) to a final concentration of 100 mU/ml

um Ringer's. Again tissue composition did not change significantly.

Whereas exposure of hemibladders incubated with lithium Ringer's mucosa and serosa to vasopressin or to cyclic AMP had little effect on SCC, exposure of the mucosal surface to amphotericin B caused a large increase in SCC (Fig. 10, Table 10*a*) as it did in the paired hemibladders bathed with sodium Ringer's. In contrast to the increased SCC, PD did not increase significantly after amphotericin B in either group, so that, in both, calculated tissue resistance fell markedly.

Amphotericin B had similar stimulatory effects on SCC, without affecting PD, when hemibladders were bathed with either lithium Ringer's mucosa, sodium Ringer's serosa or with sodium Ringer's mucosa, lithium Ringer's serosa (Fig. 11, Table 10*b*). Again, there were large decreases in tissue resistance following amphotericin B (Table 10*b*). In the experiments with amphotericin B, tissue compositions were not determined.

Effects of Absence of Serosal Medium Calcium on Transepithelial Cation Transport

The stimulatory effects of amphotericin B on transepithelial cation transport following incuba-

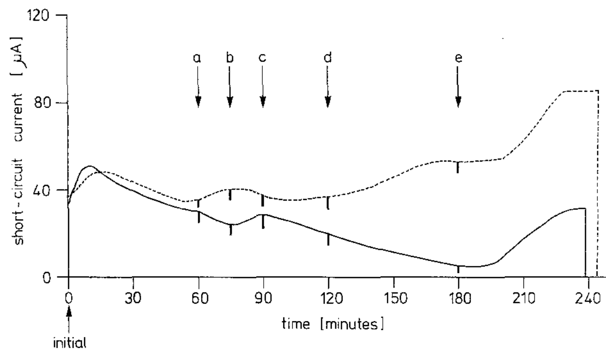

Fig. 10. Effect on SCC of amphotericin B (20 μg/ml) with lithium Ringer's or sodium Ringer's. At *a* chambers were drained and refilled five times with either sodium Ringer's (-----) or lithium Ringer's (———). These washings were repeated at *b*, *c*, and *d*. At *e* amphotericin was added to the mucosal medium to a final concentration of 20 μg/ml. (Reproduced from Macknight & Hughes, 1981, by permission of Raven Press.)

Table 10. Effects on electrical parameters of amphotericin B, 20 μg/ml[a]

Media	PD (mV)	SCC (μA)	R_t (ohm cm^2)
a) After exposure to lithium Ringer's mucosa and serosa:			
1) After 120-min incubation with lithium Ringer's–			
Li Ringer's (6)	2±1	4± 1	4700±1200
Na Ringer's (6)	23±6	42± 9	4400± 700
Δ	21±6	38± 8	400±1400
P	<0.02	<0.01	>0.70
2) After 50-min exposure to amphotericin B, mucosal medium–			
Li Ringer's (6)	3±2	24± 5	1200± 300
Na Ringer's (6)	13±5	68±10	1300± 300
Δ	10±4	44± 9	0± 200
P	<0.05	<0.01	>0.80
b) After exposure to lithium Ringer's mucosa or serosa:			
1) After 120-min incubation with lithium Ringer's–			
Li Ringer's *m* (4)	3±1	8± 1	3700± 500
Li Ringer's *s* (4)	4±1	5± 1	6700±1500
Δ	1±1	3± 1	3000±1500
P	>0.60	>0.05	>0.10
2) After 50-min exposure to amphotericin B, mucosal medium–			
Li Ringer's *m* (4)	2±1	28± 6	600± 100
Li Ringer's *s* (4)	6±1	48±11	800± 100
Δ	4±1	20±17	200± 100
P	<0.05	>0.30	>0.30

[a] Initial average values for *b*) in sodium Ringer's were 30±11 mV, 26±7 μA and 8700±1100 ohm cm^2.

tion of tissues in serosal lithium Ringer's, indicated that the inhibition of transport in the absence of serosal sodium reflected predominantly an inhibition of cation entry to the cells from the mucosal medium rather than of extrusion across the baso-

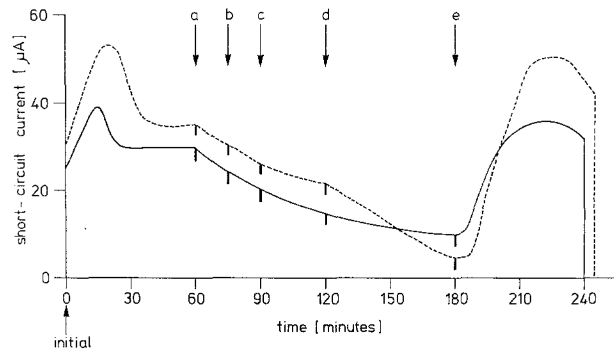

Fig. 11. Effect on SCC of amphotericin B (20 μg/ml) with either lithium Ringer's mucosa, sodium Ringer's serosa or sodium Ringer's mucosa, lithium Ringer's serosa. At *a* chambers were drained and refilled five times with either sodium Ringer's mucosa, lithium Ringer's serosa (-----) or lithium Ringer's mucosa, sodium Ringer's serosa (———). These washings were repeated at *b*, *c* and *d*. At *e* amphotericin was added to the mucosal media to a final concentration of 20 μg/ml. (Reproduced from Macknight & Hughes, 1981, by permission of Raven Press.)

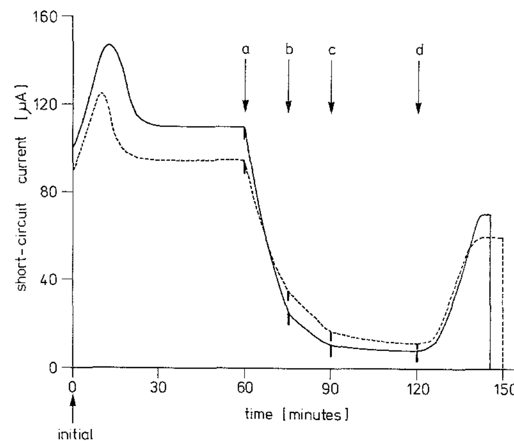

Fig. 12. Effect on SCC of calcium-free serosal medium with lithium Ringer's mucosa. At *a* chambers were drained and refilled five times with either lithium Ringer's mucosa, choline Ringer's serosa (-----) or lithium Ringer's mucosa, calcium-free choline Ringer's containing EGTA (2×10^{-3}M) serosa (———). These washings were repeated at *b* and *c*. At *d* vasopressin was added to the serosal media to a final concentration of 100 mU/ml

lateral cellular membranes. It has been suggested that the inhibition of transepithelial sodium transport which is found when sodium is replaced in the serosal medium by choline results from accumulation of cellular calcium in the absence of sodium-calcium countertransport at the basolateral membrane (Erlij & Grinstein, 1977; Taylor & Windhager, 1979). To test whether or not the inhibition of lithium transport from mucosa-to-serosa was a consequence of such an accumulation of calcium, hemibladders were incubated with lithium Ringer's mucosa and either choline Ringer's or cal-

Table 11. Effects of absence of calcium on electrical parameters during incubation in lithium Ringer's mucosa, sodium-free choline Ringer's serosa[a]

Serosal media	PD (mV)	SCC (μA)	R_t (ohm cm^2)
a) After 60 min in lithium Ringer's mucosa, choline Ringer's serosa:			
Choline Ringer's (4)	11±3	18± 4	4400± 500
Ca-free choline Ringer's +EGTA, 2×10^{-3} M (4)	3±1	11± 4	2500± 900
Δ	8±2	7± 2	1900±1300
P	<0.05	<0.05	>0.20
b) At peak of response to vasopressin:			
Choline Ringer's +vasopressin (4)	22±5	54±12	3200± 300
Ca-free choline Ringer's + EGTA + vasopressin (4)	14±4	69±10	1600± 400
Δ	8±6	15± 9	1600± 600
P	>0.20	>0.10	>0.05

[a] After initial incubation with sodium Ringer's where average values were 59±9 mV, 83±10 μA and 5900±500 ohm cm^2, one hemibladder was then incubated with lithium Ringer's mucosa, choline Ringer's serosa while the paired hemibladder was incubated with lithium Ringer's mucosa, calcium-free choline Ringer's containing EGTA, 2×10^{-3} M, serosa, After 60 min vasopressin was added to both serosal media to give a final concentration of 100 mU/ml.

cium-free choline Ringer's containing EGTA, 2×10^{-3} M serosa. The absence of medium calcium did not prevent the falls in SCC and PD which substitution of choline for serosal medium sodium also produced (Fig. 12, Table 11). Nor did the presence of calcium in the serosal medium prevent the stimulation of transport produced by vasopressin. Similar results were obtained when calcium-free choline Ringer's without EGTA was used as the serosal medium. Thus it cannot be demonstrated that an accumulation of cellular calcium is responsible for the inhibition of transepithelial lithium transport in the absence of serosal sodium. The decrease in transport with lithium Ringer's mucosa and choline Ringer's serosa (Table 11) seemed much greater than that seen with lithium Ringer's mucosa and sodium Ringer's serosa (Tables 5 & 6). This was confirmed in a separate series of experiments (Fig. 13, Table 12). It would seem, therefore, that just as for transepithelial sodium transport, serosal sodium is required to sustain transepithelial lithium transport. Under these conditions tissues gained no more lithium than with sodium Ringer's serosa (Table 13). But with choline Ringer's serosa they lost water, potassium and

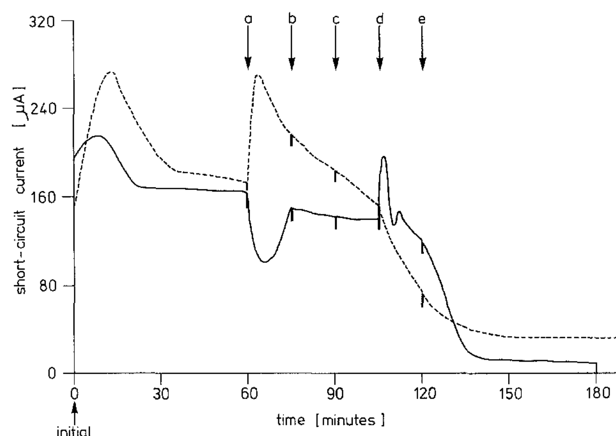

Fig. 13. Effect on SCC of choline Ringer's serosa with lithium Ringer's mucosa. At *a* chambers were drained and refilled five times with either sodium Ringer's mucosa and serosa (-----) or sodium Ringer's mucosa, choline Ringer's serosa (——). These washings were repeated at *b* and *c*. At *d* lithium Ringer's replaced sodium Ringer's as the mucosal medium for both hemibladders, and tissues were washed at *e* with media of the same composition as at *d*

Table 12. Effects of sodium-free choline Ringer's serosa on electrical parameters during incubation in lithium Ringer's mucosa[a]

Serosal media	PD (mV)	SCC (μA)	R_t (ohm cm^2)
a) After 45 min in sodium Ringer's mucosa, sodium or choline Ringer's serosa:			
Choline Ringer's (7)	50±11	86±23	5300±700
Sodium Ringer's (7)	116±32	125±23	7000±500
Δ	66±22	39± 8	1700±800
P	<0.025	<0.005	>0.10
b) After 60 min in lithium Ringer's mucosa, sodium or choline Ringer's serosa:			
Choline Ringer's (7)	5± 1	9± 1	4400±200
Sodium Ringer's (7)	22± 4	39± 8	5000±500
Δ	18±13	30± 8	500±400
P	<0.005	<0.01	>0.20

[a] After initial incubation with sodium Ringer's, one hemibladder was then incubated with sodium Ringer's mucosa and serosa, while the paired hemibladder was incubated with sodium Ringer's mucosa, choline Ringer's serosa. After 45 min the mucosal solutions were replaced by lithium Ringer's and tissues were incubated for a further 60 min before being taken for analysis of epithelial composition.

chloride. The decrease in tissue sodium was not matched by changes in water or chloride, suggesting that tissue exchanged nonunulin space sodium for serosal choline.

Table 13. Effects on epithelial noninulin space composition of sodium-free choline Ringer's bathing the serosal surface with lithium Ringer's bathing the mucosal surface[a]

Serosal medium		H_2O (kg/kg dry wt)	Noninulin space			
			Na	K	Li	Cl
			(mmol/kg dry wt)			
Choline Ringer's		2.24±0.17	49± 6	274±15	77±14	234±10
Sodium Ringer's		2.62±0.15	197±17	341±10	92±13	280±14
	Δ	0.38±0.11	148±19	67± 9	15±21	47±10
	P	<0.02	<0.001	<0.001	>0.40	<0.005

[a] Paired hemibladders were incubated as described in Table 12 which presents the averaged electrical parameters for these tissues.

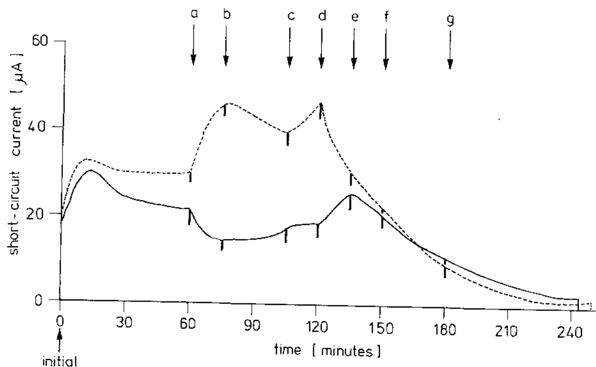

Fig. 14. Effect on SCC of high potassium media. At *a* chambers were drained and refilled five times with either sodium Ringer's mucosa and serosa (-----) or high potassium-sodium Ringer's (Na, 85 mM; K, 35 mM) mucosa and serosa (———). This washing was repeated at *b* and *c*. At *d*, media were replaced by either lithium Ringer's mucosa and serosa (-----) or high potassium-lithium Ringer's (Li, 85 mM; K, 35 mM) mucosa and serosa (———). These washings were repeated at *e*, *f* and *g*

Effects of Increased Serosal Medium Potassium Concentration on Transepithelial Cation Transport

The inhibition of transepithelial sodium and lithium transport by serosal lithium Ringer's was associated with a loss of tissue potassium (Tables 2, 4, 7*b*, 8*b*). To minimize this loss, hemibladders were incubated in mucosal and serosal media in which potassium concentration was increased to 35 mmol/liter by isosmotic substitution of potassium chloride for sodium or for lithium chloride. Following sodium Ringer's, hemibladders incubated in high potassium-sodium media had both SCC and PD depressed (Fig. 14). Substitution of lithium for sodium caused equal depression of transport in both normal and high potassium media, with transport virtually abolished by 120 min. Tissue analysis revealed that the epithelia incubated in high potassium-lithium media contained about twice the potassium content (388±14 compared with 198±8 mmol/kg dry wt) and two-thirds of the lithium content =305±26 compared with 471±25 mmol/kg dry wt) of those incubated in lithium Ringer's. Thus, preservation of a more normal cellular potassium content did not prevent the inhibition of transepithelial lithium transport by serosal lithium Ringer's. However, since the tissues in the high potassium medium also gained some water, the estimated cellular potassium concentration was not maintained at normal values but fell by about half to approximately 70 mmol/kg cellular water.

Discussion

In summary, these results show that transepithelial lithium movement occurred with lithium Ringer's bathing both surfaces, but that this movement was inhibited progressively, as was transepithelial sodium transport, by lithium Ringer's serosa. Though lithium continued to move from mucosa to serosa with sodium Ringer's serosa, this movement represented only about a half of the transepithelial sodium movement when sodium Ringer's bathed both surfaces. As with transepithelial sodium movement, transepithelial lithium movement was inhibited by mucosal amiloride and by serosal ouabain, confirming the results of others in this tissue (Herrera et al., 1971; Herrera, 1972). It was stimulated by amphotericin B with lithium Ringer's mucosa and serosa, and by vasopressin with sodium Ringer's serosa. Vasopressin also promoted transepithelial sodium movement with lithium Ringer's serosa. Measurement of noninulin space epithelial composition revealed that tissue exposed to lithium gained lithium from both mucosal and serosal media in exchange for sodium and for potassium, most of the gain occurring from the serosa.

This study of lithium transport differs from others reported in toad urinary bladder in that complete, rather than partial substitutions of lithium for sodium were made (contrast Bentley & Wasserman, 1972; Singer & Franko, 1973), and these substitutions were made with lithium as the chloride, not as the sulfate salt (contrast Herrera et al., 1971 and Herrera, 1972). Additionally, no other workers have described the composition of the epithelial cells themselves. The studies of Herrera and associates (1971, 1972) employed analysis of the whole thickness of the epithelium, and, as discussed in detail previously (Macknight et al., 1975*a*), it is not possible to draw conclusions about epithelial cell composition from such preparations, for these cells constitute only about 10 percent of the total tissue mass.

Relationship of Noninulin Space Composition to Cellular Composition

A full analysis of the effects of lithium on noninulin space composition requires a knowledge of the extent to which this composition derived from chemical analysis can be equated with the composition of the epithelial cells themselves. This question has been comprehensively reviewed recently in relation to toad bladder epithelial cells (Macknight, DiBona & Leaf, 1980). From detailed comparisons of data from isotopic studies, chemical analyses and electron microprobe analyses (Macknight, 1980), it seems that noninulin space potassium and water contents can be equated with cellular contents, whereas noninulin space sodium and chloride contents overstate the cellular contents of these ions. For sodium, most of the excess cation seems to be sequestered in a noncellular serosal compartment, and cellular sodium can be equated with sodium of mucosal origin when sodium Ringer's bathes both surfaces of the normally transporting tissue. Cell sodium approximates 20 to 40 mmol/kg dry wt under these conditions and the remainder, which varies from about 120 to 200 mmol/kg dry wt or so in different preparations, can be regarded as lying outside of the transporting epithelial cells. These considerations are important in interpreting the changes in noninulin space composition in the present experiments.

With lithium Ringer's mucosa, sodium Ringer's serosa, there was an uptake of 66 mmol/kg dry wt of lithium and a total loss of 67 mmol/kg dry wt of sodium and potassium, without change in water or chloride contents (Table 6). Only the loss of sodium was statistically significant. These changes seem to represent a new steady-state inasmuch as no further lithium was gained in four hemibladders incubated 240 min. The loss of 47 mmol/kg dry wt of sodium is in excellent agreement with the size of the cellular sodium pool determined from isotopic studies (Macknight et al., 1975*a*) and electron-microprobe experiments (Rick et al., 1978). This suggests that lithium is completely displacing cellular sodium under these conditions. In addition, it may also cause a small decrease in cellular potassium. Just as for sodium, some of the noninulin space lithium seems to be associated with the mucosal surface of the tissue rather than being truly intracellular, for about 15 to 20 mmol/kg dry wt was removed at the end of the experiment by washing the mucosal surface with choline Ringer's containing 10^{-4} M amiloride.

With sodium Ringer's serosa, amiloride abolished transepithelial PD and SCC and lithium content fell by 42 ± 8 mmol/kg dry wt (Table 7*a*). However, a residual content of lithium (11 ± 2 mmol/kg dry wt) remained even after washing of the mucosal surface with choline Ringer's with amiloride (*see* Results). This represents a cellular concentration of about 3 mmol/kg water. Apparently, the cells are unable to lower their lithium concentration as much as they can their sodium concentration after amiloride in comparable studies (Macknight et al. 1975*a*). This may reflect a lower affinity of the basolateral transport system for lithium than for sodium, an interpretation consistent with the tendency for cellular potassium to fall with mucosal lithium.

With lithium Ringer's bathing the serosal surface, both noninulin space potassium and sodium decreased (Tables 2 and 4). Since chloride and water contents were either unaltered (sodium Ringer's mucosa, Table 4) or increased (lithium Ringer's mucosa, Table 2), the lost potassium must have been replaced by lithium entering the cells. In contrast, noninulin space sodium of serosal origin seems not to be cellular (Macknight et al., 1980), and, with sodium Ringer's mucosa, the lost sodium must have exchanged with lithium in a noncellular compartment. Therefore, of the lithium gained, only about half entered the cells (Table 4). With lithium Ringer's mucosa and serosa (Table 2) some lithium will enter cells from the mucosal medium displacing sodium from the so-called transport pool. This presumably amounts to about 40 mmol/kg dry wt. Inasmuch as about 20 mmol sodium/kg dry wt remained with lithium Ringer's mucosa and serosa, the exchange of serosal lithium for noninulin space sodium seemed not to be complete. Alternatively, some or all of this residual sodium may be of mucosal origin

located in the cellular transport pool from which it cannot be extruded when lithium Ringer's (or choline Ringer's) bathes the serosal surface.

With lithium Ringer's mucosa and serosa, an additional uptake of lithium, together with chloride and water to maintain electrical and osmotic balance, was detected (Table 2) and the estimated cellular lithium content (corrected for sodium-lithium exchange in a serosal noncellular compartment) approximated 250 mmol/kg dry wt at 60 min and 275 mmol/kg dry wt at 120 min. With cellular water contents of 2.83 and 3.20 kg/kg dry wt, respectively (Table 2), cellular lithium concentrations can be estimated as 88 and 86 mM, respectively, with 117 mM lithium in both media. The tendency towards cellular swelling under these conditions indicates that a new balance is established between influx of lithium to the cells and its efflux to the extracellular fluid, and is consistent with the suggestion that the basolateral transport mechanism has a lower affinity for lithium than it does for sodium.

The uptake of lithium to the cells from the serosal medium indicates that basolateral membrane permeability to lithium is somewhat greater than to sodium. With the sodium pump inhibited by ouabain, toad bladder epithelial cells gain about 50 mmol sodium/kg dry wt from the serosal medium over 60 min and a further 80 mmol over another 180 min (Macknight et al., 1975*b*). In contrast, in the absence of ouabain, cells gained 100 mmol lithium/kg dry wt over 120 min (taken as equivalent to the loss of cellular potassium) with lithium Ringer's serosa, sodium Ringer's mucosa (Table 4). With lithium Ringer's mucosa and serosa, lithium gained from the serosa would have replaced much of the potassium lost as well as accompanying the chloride gained, and would have approximated 220 mmol/kg dry wt after 60 min and 250 mmol/kg dry wt after 120-min incubation (Table 2). Cellular lithium was not affected by ouabain under these conditions (Table 8*b*). Though lithium may enter some cells via the ouabain-sensitive (Na-K)-pump (Beaugé & Sjodin, 1968; Smith, 1974), this influx is competitively inhibited by medium potassium (Smith, 1974). With 4 mM potassium in the medium, it is unlikely that this pathway would have contributed significantly to lithium entry to the cells across the basolateral membrane. Additionally, in the absence of sodium, lithium-sodium countertransport across the basolateral membrane is excluded, and in the absence of medium bicarbonate, a bicarbonate-sensitive leak pathway, as has been described in red blood cells (Duhm & Becker, 1977; Funder, Tosteson & Wieth, 1978), is also excluded. It can be concluded, therefore, that the basolateral cellular membranes are more permeable to lithium than to sodium.

Is There a Net Flux of Lithium from Mucosa to Serosa with Lithium Ringer's Mucosa and Serosa?

With lithium Ringer's bathing both surfaces and the tissue short-circuited, there is no electrochemical potential gradient for any ion across the tissue, and, therefore, in the steady-state, no net passive flux of lithium could occur. The virtual abolition of SCC after 60 min or so (Table 1), might, therefore, reflect the situation under steady-state conditions, with the transient decrease in current from the value with sodium Ringer's to this new steady state reflecting not transepithelial lithium movement but, instead, lithium entry to the cells from the mucosal medium with displacement of sodium or potassium to the serosal medium (Herrera et al., 1971), or gain of chloride from the serosal medium. To assess this possibility, the current flow across five hemibladders when lithium Ringer's replaced sodium Ringer's was quantitated and compared with the changes in cellular composition in these same hemibladders which had occurred by the time SCC had declined to its new very low value. The mean current flow during the transient represented 365 ± 23 mmol/kg dry wt. During this period the noninulin space gained a total of 445 ± 33 mmol/kg dry wt of lithium and lost 202 ± 14 mmol/kg dry wt of sodium and 201 ± 14 mmol/kg dry wt of potassium, the excess of lithium gained over sodium and potassium lost (42 mmol) being balanced by a gain of 56 ± 25 mmol/kg dry wt of chloride. However, of this lithium gained, no more than about 70 mmol/kg dry wt can have been derived from the mucosal medium, for this was the quantity of cellular lithium with lithium Ringer's mucosa, sodium Ringer's serosa (Table 6) and also the difference in noninulin space lithium following amiloride in tissues bathed on mucosa and serosa with lithium Ringer's (Table 7*b*). Thus, only about 70 mmol/kg dry wt of the total current of 364 mmol/kg dry wt, could have been carried across the tissue by a combination of lithium entry from the mucosa and potassium exit to (and/or chloride entry from) the serosa. The remaining current must reflect a net lithium movement which, in the absence of any electrochemical gradient for lithium across the tissue, would represent an active transport of this ion. The lithium gained by the noninulin space from the serosal medium with chloride, or in exchange for sodium and potassium

lost to this medium, cannot contribute to SCC, being electrically silent.

Lithium Movement from Cell to Serosa

Under short-circuit conditions in toad bladder epithelial cells, the potential differences across both apical and basolateral membranes are oriented cell interior negative with respect to the medium, though the absolute magnitude of these potentials is yet to be firmly established (for discussion, *see* Macknight et al., 1980). With a cellular lithium concentration approximating 86 mM and a medium concentration of 117 mM, lithium will enter the cells across the apical plasma membrane down its electrochemical gradient from the mucosal medium, but will require active transport from the cells to the serosal medium. The localization of the active step at the basolateral membrane where the (Na-K)-ATPase is found in transporting epithelia (DiBona & Mills, 1979) suggests that this enzyme may be involved in lithium transport from cell to serosa. However, this was not established directly, for ouabain had no additional effects on either electrical parameters or tissue composition (Table 8*b*) with lithium Ringer's mucosa and serosa. Lithium appears to be the only species capable of being translocated in both directions by the sodium pump in its normal mode of operation, for lithium may substitute for potassium outside and for sodium inside (Dunham & Hoffman, 1978). However, the selectivity radio Na/Li for translocation from cell to medium may be as high as 10:1 (Dunham & Senyk, 1977) and it is only when cellular sodium concentrations are less than 1 mM or so that ouabain-sensitive lithium efflux can be detected (Beaugé, 1975; Dunham & Senyk, 1977). With lithium Ringer's mucosa and serosa, cellular sodium will approach this concentration. However, with sodium Ringer's mucosa, lithium Ringer's serosa, cellular sodium, concentration will be considerable greater. In a recent review, Erhlich and Diamond (1980) argued against an appreciable lithium extrusion by the (Na-K)-ATPase from erythrocytes, nerve, muscle or epithelia, ascribing the extrusion which is found to sodium-lithium countertransport. They were principally concerned, however, with the behavior of systems exposed to a low medium lithium concentration and normal medium sodium concentration. Such a countertransport mechanism can not be operating in our experiments with lithium Ringer's bathing mucosa and serosa, and, if the (Na-K)-ATPase is not involved, an alternative active transport pathway for lithium at the basolateral membrane is required.

Since lithium must be actively transported from mucosa to serosa with lithium Ringer's mucosa and serosa, the SCC with lithium Ringer's mucosa, sodium Ringer's serosa which also utilizes an amiloride and ouabain-sensitive cellular pathway, could also represent a direct active transport of lithium. However, the alternative of apical lithium entry and basolateral sodium-lithium countertransport might also contribute to the net mucosal-to-serosal lithium movement. This alternative requires that any sodium entering the cells across the basolateral membrane via this countertransport pathway would then be extruded from the cells via the ouabain-sensitive sodium pump. The inhibition of SCC by ouabain under these conditions of incubation would be indirect, and a consequence of cellular sodium accumulation decreasing the electrochemical gradient for sodium entry across the basolateral membrane.

The effects of ouabain on cell composition with lithium Ringer's mucosa, sodium Ringer's serosa (Table 8*a*) do not allow a firm distinction to be made between these alternatives. With sodium Ringer's bathing both surfaces, the epithelial cells gain sodium after ouabain predominantly from the mucosal medium though a significant amount is also derived from the serosa (Macknight et al. 1975*b*; Rick et al., 1978). With lithium substituted for mucosal sodium, cells contain significantly less lithium following exposure to ouabain (Table 8*a*). Blockage of the basolateral pump should result in increased cellular lithium if lithium entry from the mucosal medium continues unaffected. Therefore, the decreased cellular lithium under these conditions must indicate that apical membrane permeability to lithium has decreased following ouabain. A decreased apical membrane permeability to sodium follows inhibition of transepithelial sodium transport by ouabain and this has been attributed to increased cellular sodium (Erlij & Smith, 1973; Moreno et al., 1973; Lewis, Eaton & Diamond, 1976; Turnheim, Frizzell & Schultz, 1978). Cellular sodium rises after ouabain with lithium Ringer's mucosa (Table 8*a*). Since the basolateral membrane is permeable to lithium, and lithium is absent from the serosal medium, some diffusional loss of lithium from the cell would be expected. A combination of inhibition of apical membrane lithium permeabiliy and diffusional loss from cell to serosa would explain the lower cellular lithium content following ouabain. Equally, with decreased apical membrane permeability to lithium, sodium-lithium

countertransport at the basolateral membrane would lower cell lithium and contribute directly to the higher cell sodium content.

Lithium Movement from Mucosa to Cell

Whatever the detailed mechanism of lithium movement from cell to serosa across the basolateral membrane, the dominant site of inhibition of transepithelial lithium (and sodium) transport was localized to the apical cellular membrane in these experiments, for amphotericin B, which increases apical membrane permeability nonselectively in toad urinary bladder (Lichtenstein & Leaf, 1965) and in other epithelia (Nielsen, 1971; Stroup, Weinman, Hayslett & Kashgarian, 1974; Frizell & Turnheim, 1978), partially restored SCC in tissues bathed by lithium Ringer's mucosa or serosa (Table 10*b*, Fig. 11) or by lithium on both mucosa and serosa (Table 10*a*, Fig. 10). This response was accompanied by a substantial decrease in tissue resistance as expected from the marked increase in apical membrane ionic permeability. In addition, vasopressin which acts primarily to increase apical membrane sodium permeability in toad bladder (Civan & Frazier, 1968; Macknight, Leaf & Civan, 1971), also promoted SCC with sodium Ringer's mucosa, lithium Ringer's serosa (Table 9*a*, Fig. 8) and with lithium Ringer's mucosa, sodium Ringer's serosa (Table 9*b*, Fig. 9), again with substantial decreases in tissue resistance. However, with lithium Ringer's mucosa and serosa, unlike the effect of amphotericin B, there was only a very small increase in SCC after vasopressin and tissue resistance was unaltered.

The mechanism of this inhibition of sodium and lithium entry to the cells associated with lithium Ringer's serosa is unclear. It may be that it is the absence of serosal sodium, rather than the presence of serosal lithium which is responsible. It is well established that transepithelial sodium transport is partially inhibited when serosal sodium is replaced by choline Ringer's (Leaf, 1965), and substitution of choline Ringer's for serosal sodium Ringer's also depressed transepithelial lithium transport with lithium Ringer's mucosa (Table 12, Fig. 13), as Herrera et al. (1971) have also demonstrated in toad urinary bladder.

It has been argued that the depression of SCC in the absence of serosal sodium results from cellular accumulation of calcium as a consequence of inhibition of sodium-calcium countertransport across the basolateral cellular membrane (Erlij & Grinstein, 1977; Taylor & Windhager, 1979). The resulting increase in cellular calcium is thought to inhibit sodium entry to the cells across the apical membrane. No evidence for this hypothesis was obtained, in that the absence of serosal medium calcium did not prevent the decline in SCC with lithium Ringer's mucosa, choline Ringer's serosa (Table 11, Fig. 12), and vasopressin still resulted in a marked stimulation of SCC under these conditions.

It has also been suggested that decreased cellular potassium may inhibit sodium entry to the cells from the mucosal medium (Robinson & Macknight, 1976). Though cellular potassium decreased after serosal sodium was replaced by lithium (Tables 2 & 4), incubation in a medium with increased potassium concentration, though it raised potassium content appreciably and minimized lithium accumulation, did not prevent the inhibition of SCC (Fig. 14). This finding argues against a central role of a change in cellular potassium in decreasing apical membrane lithium and sodium permeability. However, it must be appreciated that inasmuch as some cellular swelling results from incubation in media with high potassium concentration, the cellular concentration of potassium was not maintained at normal levels in this experimental situation.

Changes in cellular volume in epithelia may also play an important role in regulating apical membrane permeability. Decreased volume is associated with a decreased SCC, whereas increased volume results in stimulation of transport (MacRobbi & Ussing, 1961; Ussing, 1965; Lipton, 1972; Bentley, Candia, Parisi & Saladino, 1973). With lithium Ringer's serosa, there was no detectable change in volume when sodium was the mucosal cation (Table 4) and there was a small increase in volume with mucosal lithium (Table 2). Thus, the decreased transport could not be accounted for by cellular shrinkage.

An attractive hypothesis is that cellular lithium itself inhibits transepithelial cation transport by inhibiting both lithium and sodium entry to the cells from the mucosal medium. This has been claimed for cell sodium in relation to sodium entry to 'tight' epithelia (Lewis et al., 1976; Cuthbert & Shum, 1977; Turnheim et al., 1978). The mechanism of sodium entry across the apical membrane has been studied extensively. This entry, though passive, involves interaction with a membrane component, which has been described in terms of a carrier-type mechanism (Frazier, Dempsey & Leaf, 1962) or as an ion-selective membrane channel (Fuchs, Larsen & Lindemann, 1977). Dis-

tinction between these two alternatives remains to be accomplished (Schultz, Thompson & Suzuki, 1981). However, whatever the nature of the underlying mechanism, it has been established that sodium and lithium utilize the same amiloride-sensitive pathway for entry to the cells from the mucosal medium in 'tight' epithelia (Biber & Curran, 1970; Herrera, 1972; Leblanc, 1972; Nagel, 1977; Thompson & Dawson, 1978; Benos, Mandel & Simon, 1980; Biber & Mullen, 1980*a, b*). Interactions between cellular sodium or lithium and the entry mechanism may occur, and affect the availability of entry sites, thus inhibiting cation entry to the cells. Inhibition of lithium transport may also be contributed to by a decreased electrochemical gradient driving lithium into the cells from the mucosal medium [a consequence both of the increased cellular lithium concentration and also of the decreased electrical potential gradient at this membrane (Nagel, 1977)]. However, decreased driving force alone cannot provide a complete explanation for the inhibition of lithium transport, since amphotericin B, which affects only passive permeability, resulted in a large increase in current under conditions where the transepithelial transport had been virtually abolished.

Both cellular sodium and lithium therefore, could compete for and block the sites used by sodium and lithium for entry across the apical membrane. Normally, since the basolateral membrane permeability to sodium is relatively very low, cellular sodium is maintained at a low concentration by the continued activity of the sodium pump and the inhibition of sodium entry by cellular sodium is minimized. However, basolateral membrane permeability to lithium exceeds that to sodium and, with lithium Ringer's serosa, lithium accumulates intracellularly, thus inhibiting entry of both mucosal sodium and mucosal lithium. This inhibition of lithium entry was not overcome by vasopressin or by cyclic AMP, both of which are thought to increase the number of cation-specific (and, therefore, lithium-sensitive) entry sites in the apical membrane; but it was overcome by amphotericin B, which creates nonselective ionic channels in the apical membrane.

In summary, lithium, like sodium, is actively transported across toad urinary bladder epithelial cells through an amiloride- and ouabain-sensitive pathway. Both sodium and lithium transport are inhibited progressively and virtually completely by serosal lithium Ringer's. Though the affinity of the sodium pump for lithium seems to be less than for sodium, this inhibition results not from inhibition of the sodium pump but from inhibition of sodium and lithium entry possibly as a consequence of the elevated cellular lithium competing for and blocking the specific apical membrane entry sites utilized both by sodium and by lithium ions.

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