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Structural and functional response of toad urinary bladder to LiCl

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Structural and functional response of toad urinary bladder to LiCl. The physiological and morphological response of toad urinary bladder was examined during mucosal exposure of LiCl both with and without vasopressin (VP). With 20 or 100 mU/ml of VP in the serosal bath there was a decrease in J_V between the first and second VP stimulation in LiCl-treated bladders (VP_{20}, $-14 \pm 6\%$; VP_{100}, $-16 \pm 5\%$) that was not different from that observed without LiCl (VP_{20}, $-8 \pm 3\%$, P = NS). However, with 1 mU/ml of VP, a significant decrease in J_V was evident in LiCl-treated ($-30 \pm 10\%$) versus control sacs ($+6 \pm 8\%$; $P < 0.02$). At all VP concentrations tested, a significant decrease in SCC and PD was observed between the first stimulation without LiCl and the second stimulation with LiCl. Both osmotic (P_f) and diffusional water permeability (P_d) were increased significantly with 11 mM LiCl only, while neither basal nor VP-stimulated urea permeability (P_u) was affected. Morphological changes paralleled the physiological alterations induced by LiCl. These data demonstrate that LiCl interferes with the osmotic response of the toad bladder to low concentrations of VP, and increases both P_f and P_d while leaving P_u unaffected. These findings coupled with the cell swelling and intracellular vacuolization suggest the presence of a defect in transepithelial water movement somewhere beyond the apical membrane of the granular cell exposed to LiCl.

Réponse structurelle et fonctionnelle de la vessie de crapaud au LiCl. La réponse physiologique et morphologique de la vessie de crapaud a été examinée pendant exposition de la muqueuse à du LiCl en présence ou en l'absence de vasopressine (VP). Pour 20 ou 100 mU/ml de VP dans le bain séreux, il y avait une diminution de J_V entre la première et la seconde stimulation par VP dans les vessies traitées par le LiCl (VP_{20}, $-14 \pm 6\%$; VP_{100}, $-16 \pm 5\%$), qui n'étaient pas différentes de celles observées sans LiCl (VP_{20}, $-8 \pm 3\%$; P = NS). Cependant, avec 1 mU/ml de VP, une diminution significative de J_V était évidente dans les sacs traités au LiCl ($-30 \pm 10\%$) par rapport aux sacs contrôles ($+6 \pm 8\%$; $P < 0{,}02$). Pour toutes les concentrations de VP testées, une diminution significative du SCC et de PD a été observée entre la première stimulation sans LiCl, et la seconde stimulation avec LiCl. Les perméabilités osmotiques (P_f) et diffusionnelles (P_d) à l'eau étaient augmentées significativement avec 11 mM de LiCl seulement tandis que la perméabilité à l'urée basale ou stimulée par la VP (P_u) n'était pas affectée. Des modifications morphologiques allaient de pair avec les altérations physiologiques induites par le LiCl. Ces données démontrent que LiCl interfère avec la réponse osmotique de la vessie de crapaud pour de faibles concentrations de VP, augmente P_f et P_d, mais laisse P_u inchangé. Ces résultats, couplés avec le gonflement cellulaire et la vacuolisation intracellulaire suggèrent la présence d'un défaut du mouvement transépithélial d'eau quelque part au delà de la membrane apicale de la cellule granulaire exposée au LiCl.

Several studies have evaluated the effects of LiCl salt on VP-induced osmotic water flow (J_V) in toad urinary bladder. Although an inhibitory effect on J_V has been demonstrated consistently in experimental animals [1–3] and humans [1, 4], conflicting results have been obtained in toad urinary bladder epithelium, a model of the collecting tubule in the mammalian nephron. Singer, Rotenberg, and Puschett [4] and Singer and Franko [5] demonstrated a significant inhibition of VP-induced J_V after exposure of the mucosal surface to LiCl. In contrast, Bentley and Wasserman [6] failed to find such an inhibitory effect at either similar or higher mucosal concentrations of LiCl. Finally, Harris and Jenner [7] reported a significant inhibition of the hydro-osmotic effect of VP after serosal exposure of toad urinary bladder to LiCl. In view of these conflicting data, the effects of LiCl on VP-induced J_V were re-examined and correlated with the morphological changes observed in toad urinary bladder epithelium. In addition, the effects of mucosal LiCl on osmotic (P_f) and diffusional (P_d) water permeability and diffusional urea permeability (P_u) were also evaluated.

Methods

The toad, *Bufo marinus*, was used in all experiments. Animals were sacrificed by double pithing and their paired urinary hemibladders were excised and rinsed in aerated amphibian Ringer's solution with the following composition in mmoles/liter: sodium chloride, 111; potassium chloride, 3.4; sodium bicarbonate, 4.0; calcium chloride, 2.7, and dextrose, 5.0. The pH of the solution in equilibrium with room air was 7.8 to 8.1, and the osmolality was 226 to 232 mOsm/kg H_2O. A slightly different composition of amphibian Ringer's solution was employed in those studies examining osmotic permeability (P_f), diffusional water permeability (P_d), and urea permeability (P_u). The second solution had the following composition in mmoles/liter; sodium chloride, 110; potassium chloride, 5; Tris-HCl, 5; calcium chloride, 1; and glucose, 5. The pH of the solution was 8.0 to 8.1, and the osmolality was 233 mOsm/kg H_2O.

Tissue preparation. For all experiments in which both physiologic and morphologic observations were made, each hemibladder was mounted as a sac on the end of a glass or plastic cannula with the mucosal surface facing inward [8] unless otherwise specified.

Physiologic studies. To investigate the effects of LiCl on VP-stimulated J_V, sacs from 16 hemibladders were filled with

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approximately 2 ml of amphibian Ringer's solution diluted 1:3.5 with distilled water (osmolality: 65 mOsm/kg H_2O) and immersed in a bath of 20 ml of aerated Ringer's solution (osmolality: 226 to 232 mOsm/kg H_2O). Potential difference (PD), short circuit current (SCC), and resistance (R) were determined every 30 min during a 90-min equilibration period (Basal I) [9]. Sacs with a PD less than 20 mV were discarded. J_V was determined gravimetrically at 10- or 30-min intervals during the same period [8]. Thereafter, all sacs were challenged with 20 mU/ml of VP (Pitressin, Parke-Davis Co., Detroit, Michigan) (Challenge I) added to the serosal bath; J_V was again determined gravimetrically after either 10 or 30 min of VP stimulation. After initial exposure to VP, all sacs were transferred to fresh Ringer's solution without VP and an additional exchange was performed 30 min later. J_V, PD, SCC, and R were measured 30 and 60 min after VP removal. After the 60-min rinse, the mucosal solution of each experimental sac ($N = 8$) was discarded and replaced with 2 ml of Ringer's solution containing 11 mM LiCl (osmolality: 65 mOsm/kg H_2O). The mucosal solution of the corresponding control sacs ($N = 8$) was exchanged for amphibian Ringer's solution (osmolality: 65 mOsm/kg H_2O). PD, SCC, and R were measured in all sacs 30 and 60 min after the addition of LiCl to the experimental sacs (Basal II). J_V was measured at 10- or 30-min intervals. At the end of this period, all sacs were exposed a second time to 20 mU/ml of VP (Challenge II) and J_V was measured at 10- or 30-min intervals up to 30 min.

The same experimental protocol was followed in two additional studies with the exception that the concentration of VP was either increased to 100 mU/ml ($N = 10$ pairs) or decreased to 1 mU/ml ($N = 9$ pairs). LiCl concentration was measured by atomic absorption spectrophotometry in Ringer's solutions and in mucosal samples obtained from experimental and control sacs at the end of each experiment.

In an additional experiment the osmotic permeability (P_f) was determined at 4.3 atm as described previously [10] in the presence of LiCl. The coefficient of osmotic permeability, P_f, in μm/sec was derived from the formula:

$$P_f = L_p RT/\bar{V}_w \qquad (1)$$

where L_p is the hydraulic conductivity, R and T have their usual meaning and $\bar{V}_w$ is the partial molar volume of water. The area of the hemibladders was calculated by weight assuming a perfect sphere. Diffusional water permeability (P_d) was determined in everted hemibladders as previously described [10] with the mucosal bath stirred at 650 rpm. Urea permeability was measured by adding ^{14}C-urea (1 μCi/ml) to the mucosal bath and sampling both serosal and mucosal baths after 30 min. Radioactive samples were added to 10 ml of Aquasol (New England Nuclear, Boston, Massachusetts) for scintillation counting in a 1215 Rackbeta (LKB Instruments, Rockville, Maryland). Standard corrections for efficiency and quenching were used. Coefficients of diffusional water permeability (P_d) in μm/sec and urea permeability (P_u) in nm/sec were computed from the Fick equation:

$$P_i = J_i^{*M \to S}/\Delta C_i \qquad (2)$$

where $J_i^{M \to S}$ is the mucosa-to-serosa radioisotopic flux, ΔC_i is the mean concentration gradient of the radioisotope and A is the surface area derived from the volume.

Morphologic studies. Tissue was prepared for light microscopy and transmission (TEM) and scanning electron microscopy (SEM) immediately after completion of the physiologic observations. Individual sacs were fixed for 30 min by immersion in a 1.3% glutaraldehyde solution containing 0.05 M sodium cacodylate (pH 7.4; osmolality, 220 to 230 mOsm/kg H_2O), the osmolality of which matched that of the serosal bathing solution. After fixation the tissue was rinsed in the buffer of identical osmolality and stored at 4°C before processing. Tissue for light microscopy and TEM was postfixed for 1 hr in 1% OsO_4 buffered with s-collidine (osmolality: 190 mOsm/kg H_2O), dehydrated through a graded series of ethanols, and infiltrated and embedded in epoxy resin (Epon) [11]. Survey sections 1 μ in thickness were cut and stained with toludine blue for light microscopy. Thin sections for TEM were cut with diamond knives, doubly stained with uranyl acetate and lead citrate and examined and photographed with an electron microscope (Zeiss 10A, Zeiss, West Germany). Tissue for SEM underwent dehydration and critical-point drying as described previously [12]. Specimens were mounted on aluminum stubs before coating to a thickness of approximately 100 Å in a Hummer V sputter coater (Technics, Inc., Alexandria, Virginia) with gold-palladium. All specimens were examined and photographed with an "Autoscan" scanning electron microscope (ETEC Corp., Hayward, California) operating at 10 kV. To ensure unbiased interpretation, each sac preparation was coded so that morphologic examination could be performed without knowledge of the experimental condition.

To provide a semiquantitative assessment of the light microscopic findings, two random tissue sections from five or six bladders in each of the seven experimental conditions were evaluated for the presence of intercellular space expansion, cell swelling, and intracellular vacuoles using a 0 to 4$^+$ grading system. All evaluations were performed without knowledge of the experimental condition.

Calculations. The percent change (Δ%) in J_V, PD, and SCC between the first (VP_1) and second (VP_2) vasopressin stimulation in control and experimental sac preparations was evaluated as follows:

$$\text{Control } (\Delta\%) = \frac{(X\ VP_2 - X\ \text{Ringer's}) - (X\ VP_1 - X\ \text{Basal})}{(X\ VP_1 - X\ \text{Basal})} \times 100 \qquad (3)$$

$$\text{Experimental } (\Delta\%) = \frac{(X\ VP_2 - X\ \text{LiCl}) - (X\ VP_1 - X\ \text{Basal})}{(X\ VP_1 - X\ \text{Basal})} \times 100 \qquad (4)$$

where X represents the corresponding value for J_V, PD, or SCC. The surface area was calculated assuming the hemibladders were spheres where the volume equals $4/3\pi r^3$ and the surface area equals $4\pi r^2$; r is the radius in centimeters.

Statistical analyses. Data were analyzed by paired and unpaired Student's t tests [13]. For examination of differences between percent changes which did not conform to a normal distribution, Mann-Whitney and Wilcoxon rank sum tests were used [13]. Differences with a probability level of less than 0.05 were considered statistically significant.

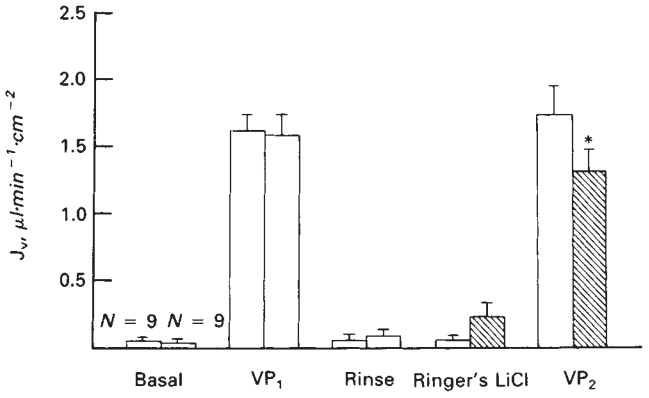


Fig. 1. *Effects of mucosal LiCl (▨ 11 mM) on J_v in the presence of 1 mU/ml of VP.* All values represent mean ± SEM.

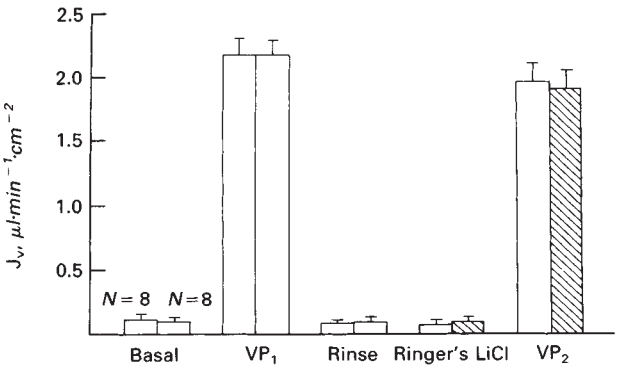


Fig. 2. *Effects of mucosal LiCl (▨ 11 mM) on J_v in the presence of 20 mU/ml of VP.* All values represent mean ± SEM.

Results

Physiologic observations

The effect of 11 mM LiCl on J_V in the presence of 1 mU/ml of VP is depicted in Figure 1. During basal conditions no significant difference in J_V was observed between the control (0.05 ± 0.01 $\mu l \cdot min^{-1} \cdot cm^{-2}$) and the experimental (0.03 ± 0.01 $\mu l \cdot min^{-1} \cdot cm^{-2}$) groups. However, J_V was enhanced after exposure to 1 mU/ml of VP in both the control (1.62 ± 0.19 $\mu l \cdot min^{-1} \cdot cm^{-2}$, $P < 0.001$) and the experimental (1.58 ± 0.13 $\mu l \cdot min^{-1} \cdot cm^{-2}$, $P < 0.001$) groups. Water flow returned to basal levels 60 min after removal of the hormone. Subsequent exposure to 11 mM LiCl slightly increased J_V, but the difference did not achieve statistical significance. However, after exposure to 1 mU/ml of VP, J_V was significantly inhibited in the presence of 11 mM LiCl when compared to values achieved in the control group (1.32 ± 0.15 $\mu l \cdot min^{-1} \cdot cm^{-2}$ vs. 1.75 ± 0.15 $\mu l \cdot min^{-1} \cdot cm^{-2}$, $P < 0.02$). Furthermore, the percentage decrease in J_V from the first to the second VP stimulation in LiCl-treated hemibladders was (−) 30.2 ± 10.0%, a value significantly different from the (+) 6.0 ± 8.0% increase observed in the control preparations ($P < 0.01$). The initial mucosal LiCl concentration was 12.9 ± 0.1 mM, while experimental sacs had a final mucosal LiCl concentration of 13.8 ± 0.1 mM.

Figure 2 illustrates the effect of 11 mM LiCl on J_V in the presence of 20 mU/ml of VP. Again, no significant difference in J_V was observed between the control and the experimental groups during basal conditions. The increase in J_V in both the control (2.20 ± 0.15 $\mu l \cdot min^{-1} \cdot cm^{-2}$, $P < 0.001$) and the experimental (2.20 ± 0.14 $\mu l \cdot min^{-1} \cdot cm^{-2}$, $P < 0.001$) sacs with VP returned to basal levels 60 min after VP removal. Exposure to 11 mM LiCl had no measurable effect on J_V. No difference was evident in the response of the control and the LiCl-treated preparations to the first and second challenges with VP [Δ% = (−) 8.3 ± 2.7 vs. (−) 13.1 ± 5.7; *P*, NS]. The initial mucosal LiCl concentration was 11.4 ± 0.8 mM. Experimental sacs had a final concentration of 13.2 ± 0.5 mM.

The effects of 11 mM LiCl on VP-stimulated J_V were also evaluated at a VP concentration of 100 mU/ml. The enhancement in J_V (2.46 ± 0.14 $\mu l \cdot min^{-1} \cdot cm^{-2}$) was significantly greater than that induced by 20 mU/ml of VP (2.21 ± 0.13 $\mu l \cdot min^{-1} \cdot cm^{-2}$, $P < 0.01$) in the presence of the same osmotic gradient (Fig. 3). As in the previous experiments, 11 mM LiCl did not alter basal J_V. In the presence of 11 mM LiCl, no significant difference in J_V was observed between sacs exposed to 100 mU/ml versus 20 mU/ml of VP. Additionally, the percentage decrease in J_V from the first to the second stimulation with VP in sacs exposed to 100 mU/ml of the hormone [Δ% = (−) 15.6 ± 5.1] was not different from that of sacs exposed to 20 mU/ml of the hormone [Δ% = (−) 15.6 ± 5.7]. In the initial amphibian Ringer's solution the LiCl concentration was 11.4 ± 1.0 mM. Sacs treated with LiCl in the presence of 100 mU/ml of VP had a final LiCl concentration of 14.8 ± 0.6 mM, not significantly different from 14.1 ± 0.5 mM measured in the presence of 20 mU/ml of VP.

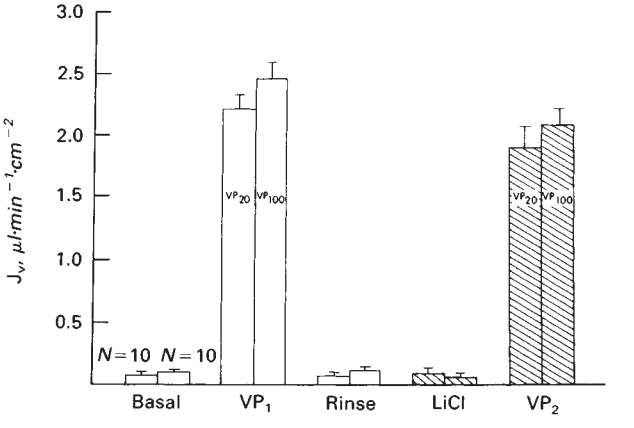


Fig. 3. *Effects of mucosal LiCl (▨ 11 mM) on VP-induced J_v.* All values represent mean ± SEM. Vasopressin (VP) quantities are: VP_{20} = 20 mU · ml^{-1} and VP_{100} = 100 mU · ml^{-1}.

Effects of mucosal LiCl on VP-stimulated PD, SCC, and R (Table 1)

At a VP concentration of 1 mU/ml, 11 mM LiCl significantly inhibited the characteristic increase in PD and SCC observed

Table 1. Changes in VP-induced PD and SCC in control and LiCl-treated toad urinary bladders

	Change, %[a]		
	VP_1	vs.	$LiCl + VP_1$
PD	+17.6 ± 14.7		−46.0 ± 11.1[b]
SCC	+18.4 ± 18.1		−47.2 ± 12.3[b]
	VP_{20}	vs.	$LiCl + VP_{20}$
PD	+28.1 ± 10.6		−14.4 ± 18.4[b]
SCC	+30.0 ± 18.4		−50.5 ± 13.4[b]
	$LiCl + VP_{20}$	vs.	$LiCl + VP_{100}$
PD	−23.8 ± 3.8		−18.5 ± 8.9 NS
SCC	−45.0 ± 7.0		−51.7 ± 6.6 NS

Abbreviations: NS, not significant; PD, potential difference; SCC, short circuit current; VP, vasopressin.

[a] The value is expressed as the percent difference in PD or SCC between the first and the second stimulation with VP. All values represent mean ± SEM.

[b] $P < 0.05$.

Table 2. Measurements of J_v, P_f, P_d, and P_u in hemibladders treated with LiCl or LiCl and VP[a]

	J_v $\mu l/cm^2/hr$	P_f $\mu m/sec$	P_d $\mu m/sec$	P_u nm/sec
Control	4.1 ± 2.1	3.5 ± 1.8	0.9 ± 0.1	14 ± 4
vs.				
LiCl, 11 mM	7.1 ± 2.2	6.3 ± 1.9	1.8 ± 0.2	16 ± 5
	$N = 12$	$N = 12$	$N = 8$	$N = 11$
	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P > 0.2$
VP, 20 mU/ml	231 ± 17	204 ± 15	6.5 ± 0.2	372 ± 21
vs.				
LiCl and VP	228 ± 11	201 ± 10	6.5 ± 0.3	330 ± 44
	$N = 8$	$N = 8$	$N = 6$	$N = 18$
	$P > 0.2$	$P > 0.2$	$P > 0.2$	$P > 0.05$

Abbreviations: J_v, osmotic water flow; P_f, osmotic permeability; P_d, diffusional water permeability; P_u, urea permeability; VP, vasopressin.

[a] The values are mean ± SEM.

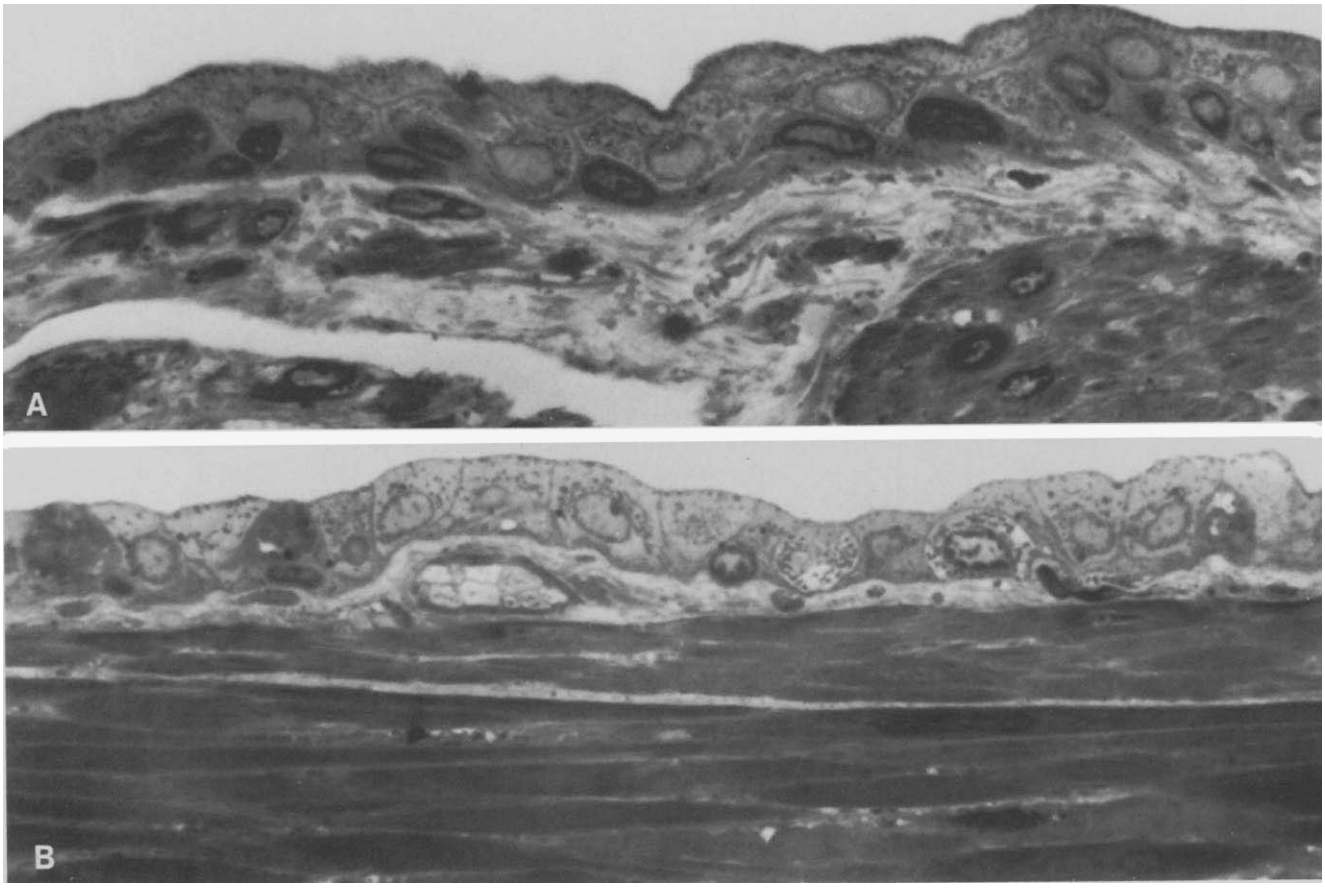


Fig. 4. *Photomicrographs of toad bladder epithelium.* **A** *Bathed in Ringer's solution in the presence of an osmotic gradient (dilute mucosa).* No intercellular spaces or intracellular vacuoles are evident. **B** *Exposed to mucosal 11 mM LiCl-Ringer's solution in the presence of an osmotic gradient.* Again no intercellular spaces or intracellular vacuoles are seen. A slight swelling of granular cells is observed. (×1100)

with VP stimulation. However, transepithelial electrical resistance remained unchanged (570 ± 70 Ω-cm^2 vs. 670 ± 123 Ω-cm^2). In the control group PD and SCC were significantly higher after the second challenge with VP and no significant difference in resistance was detected (520 ± 63 Ω-cm^2 vs. 590 ± 123 Ω-cm^2). Baseline values for PD, SCC, and R were not

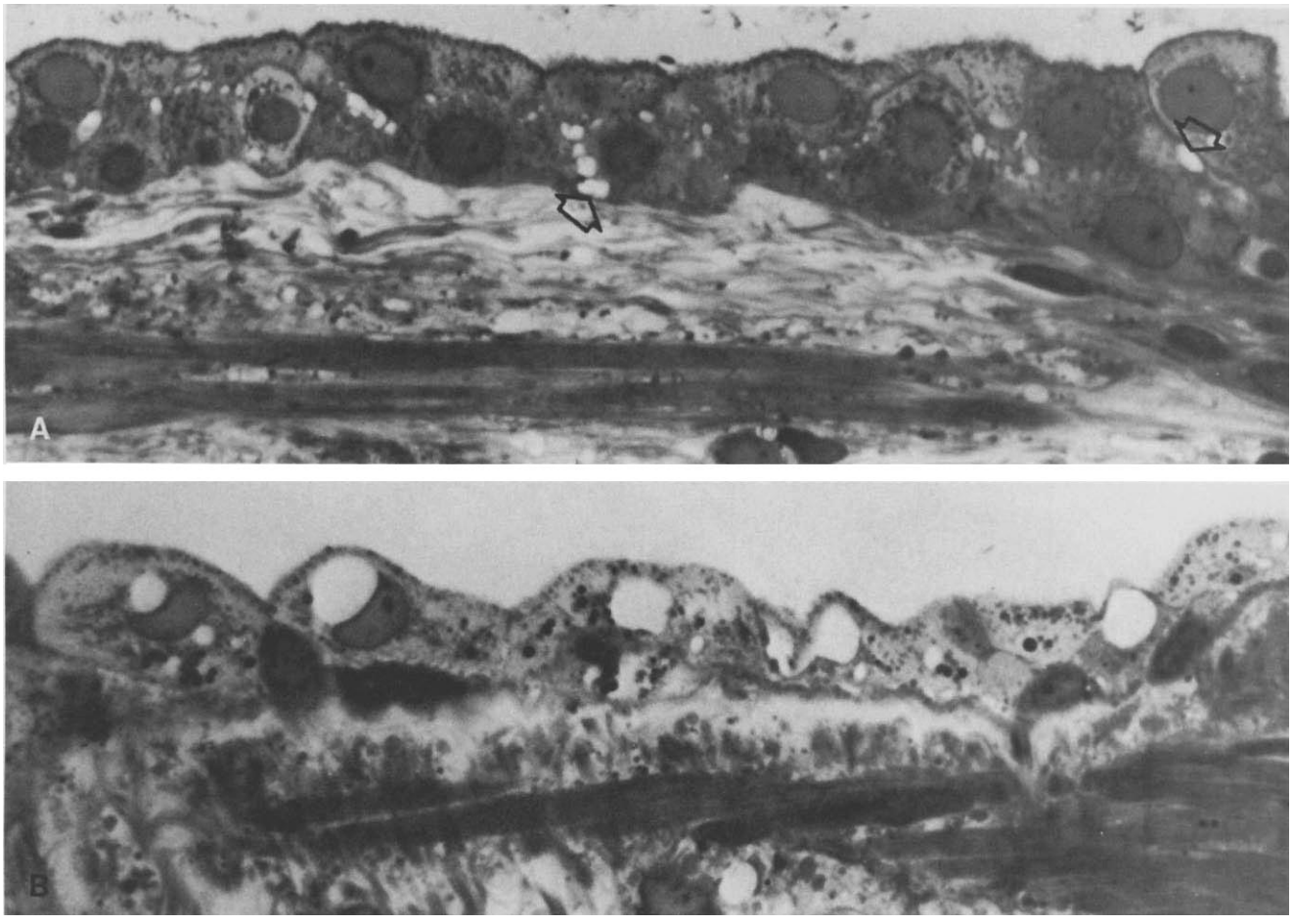


Fig. 5. *Photomicrographs of toad bladder.* **A** *Incubated with 1 mU/ml of VP in the presence of an osmotic gradient without LiCl.* Dilated intercellular spaces (*arrows*) were visible. **B** *Incubated under similar conditions as Figure 5A, but with mucosal solution replaced with 11 mM LiCl-Ringers.* Intracellular vacuolization and cell swelling were more prominent than in cells exposed to VP alone. (×1100)

significantly different between control (1 mU/ml VP: PD = 45 ± 6 mV; SCC = 47 ± 8 μAmp; R = 1033 ± 135 Ω-cm^2) and experimental preparations (1 mU/ml VP + 11 mM LiCl: PD = 40 ± 5 mV; SCC = 41 ± 7 μAmp; R = 1144 ± 238 Ω-cm^2). In addition, no significant difference was detected between the surface area of control (10.6 ± 0.7 cm^2) and experimental hemibladders (11.5 ± 0.6 cm^2).

With 20 mU/ml of VP, 11 mM LiCl significantly inhibited PD and SCC compared to the first challenge with VP measured in the absence of LiCl. Following VP stimulation transepithelial electrical resistance in the LiCl-treated hemibladders was elevated when compared to that observed with the first VP exposure (VP_1: 950 ± 148 Ω-cm^2; VP_2: 1350 ± 219 Ω-cm^2; $P < 0.05$). In controls PD and SCC were enhanced and remained unaltered after the second VP stimulation. Mucosal 11 mM LiCl also inhibited PD and SCC of hemibladders challenged with 100 mU/ml of VP. In addition, R was increased significantly in LiCl-treated sacs during the second stimulation with both 100 mU/ml (750 ± 146 Ω-cm^2 vs. 1160 ± 190 Ω-cm^2, $P < 0.01$) and 20 mU/ml (900 ± 278 Ω-cm^2 vs. 1240 ± 190 Ω-cm^2; $P < 0.05$) of VP. Baseline values for PD, SCC, and R were not different in sacs exposed to 20 mU/ml of VP (PD = 54 ± 7 mV; SCC = 40 ± 6 μAmp; R = 1475 ± 163 Ω-cm^2) vs. 20 mU/ml + 11 LiCl (PD = 52 ± 8 mV; SCC = 39 ± 6 μAmp; R = 1340 ± 250 Ω-cm^2). Comparable baseline values were recorded in sacs exposed to 100 mU/ml + 11 mM LiCl (PD = 65 ± 7 mV; SCC = 47 ± 7 μAmp; R = 1256 ± 103 Ω-cm^2). The average surface area of the hemibladders in each group was not different (VP_{20} = 8.4 ± 0.6 cm^2; VP_{20} + 11 mM LiCl = 7.9 ± 0.5 cm^2; and VP_{100} + 11 mM LiCl = 9.0 ± 0.3 cm^2).

Effects of mucosal LiCl on osmotic permeability and diffusional water and urea permeability (Table 2)

J_V, P_f, and P_d increased significantly over control values in the presence of 11 mM LiCl applied to the mucosal surface in the absence of VP, but P_u was unaffected. On the other hand, no significant differences in J_V, P_f, P_d, or P_u were detected between control sacs treated with 20 mU/ml of VP only and experimental hemibladders exposed to both VP and LiCl. The results suggest that LiCl at a concentration of 11 mM is capable of increasing both P_f and P_d when applied to the mucosal surface of the toad urinary bladder.

Morphologic observations

With light microscopy the appearance of toad bladder epithelium fixed under control conditions (Fig. 4A) and after exposure

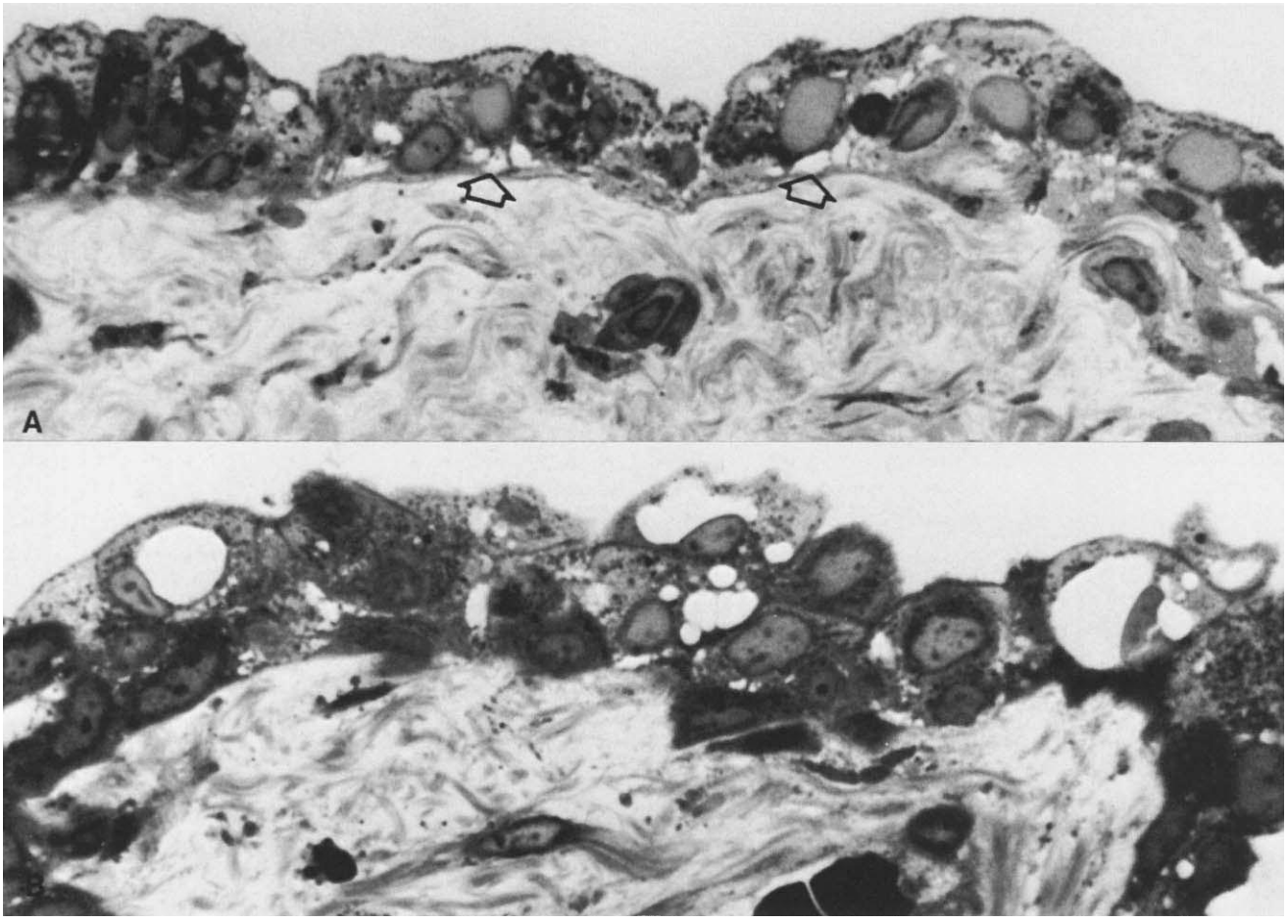


Fig. 6. *Photomicrographs of toad bladder epithelium.* **A** *Fixed after exposure to 20 mU/ml of VP in the presence of an osmotic gradient.* Intercellular spaces (*arrows*) are markedly dilated and swelling of the granular cells is also observed. **B** *Incubated in 20 mU/ml of VP in the presence of mucosal 11 mM LiCl and an osmotic gradient.* Intracellular vacuolization is striking, while intercellular space dilatation and granular cell swelling are still evident. (×1100)

Table 3. Semiquantitative assessment of light microscopic histology

Experimental condition	N[a]	Intercellular space dilatation	Intracellular vacuolization	Cell swelling
Control	(5)	0	0	0
11 mM LiCl	(6)	0	0	+
VP, 1 mU/ml	(6)	++	+	++
11 mM LiCl + VP_1	(6)	++	++	++
VP, 20 mU/ml	(6)	++	++	++
11 mM LiCl + VP_{20}	(6)	++	+++	+++
11 mM LiCl + VP_{100}	(6)	++	++++	+++

[a] The number in parenthesis indicates the number of hemibladders examined.

to 11 mM LiCl in Ringer's solution on the mucosal surface (Fig. 4B) was nearly identical. There was no intracellular vacuolization of the granular cells or enlargement of intercellular spaces after exposure to LiCl. A modest swelling of granular cells, however, was observed consistently in all bladders exposed to LiCl.

Exposure of 1 mU/ml of VP in the presence of an osmotic gradient resulted in swelling of the granular cells, discreet dilatation of the intercellular spaces, and occasional intracellular vacuolization (Fig. 5A). These changes were consistent with the increase in J_V over control values that were measured in hemibladders exposed to VP. Figure 5B illustrates the appearance of epithelium fixed after incubation with 11 mM LiCl-Ringer's solution applied to the mucosal surface before stimulation with 1 mU/ml of VP. In comparison with epithelium exposed only to VP, the granular cells contained numerous intracellular vacuoles. Figure 6A illustrates the appearance of tissue exposed to 20 mU/ml of VP in the presence of an osmotic gradient. As anticipated, intercellular space enlargement and cell swelling were prominent and similar to tissue exposed to 1 mU/ml of VP under the same physiological conditions. With the addition of 11 mM LiCl to the mucosal surface before stimulation with 20 mU/ml of VP, swelling of granular cells was increased and intracellular vacuolization was clearly more prominent (Fig. 6B). Intracellular vacuolization continued to increase in those preparations exposed to 100 mU/ml of VP in the presence of 11 mM LiCl. These findings are consistent with the inability of LiCl to inhibit the stimulation in J_V induced by either 20 or 100 mU/ml of VP. The results are summarized in Table 3 where the semiquantitative evaluation of the light microscopic findings is shown.

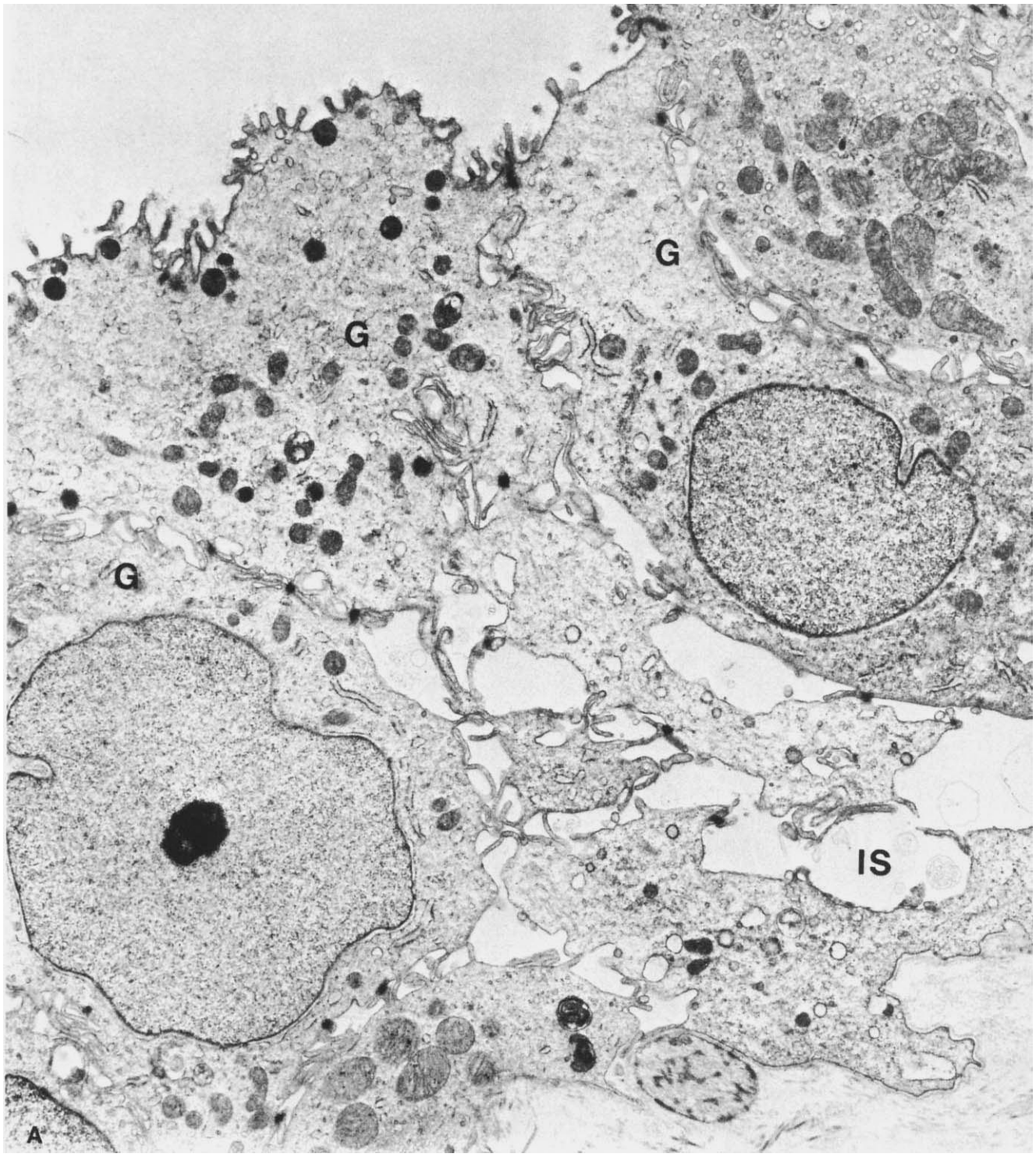
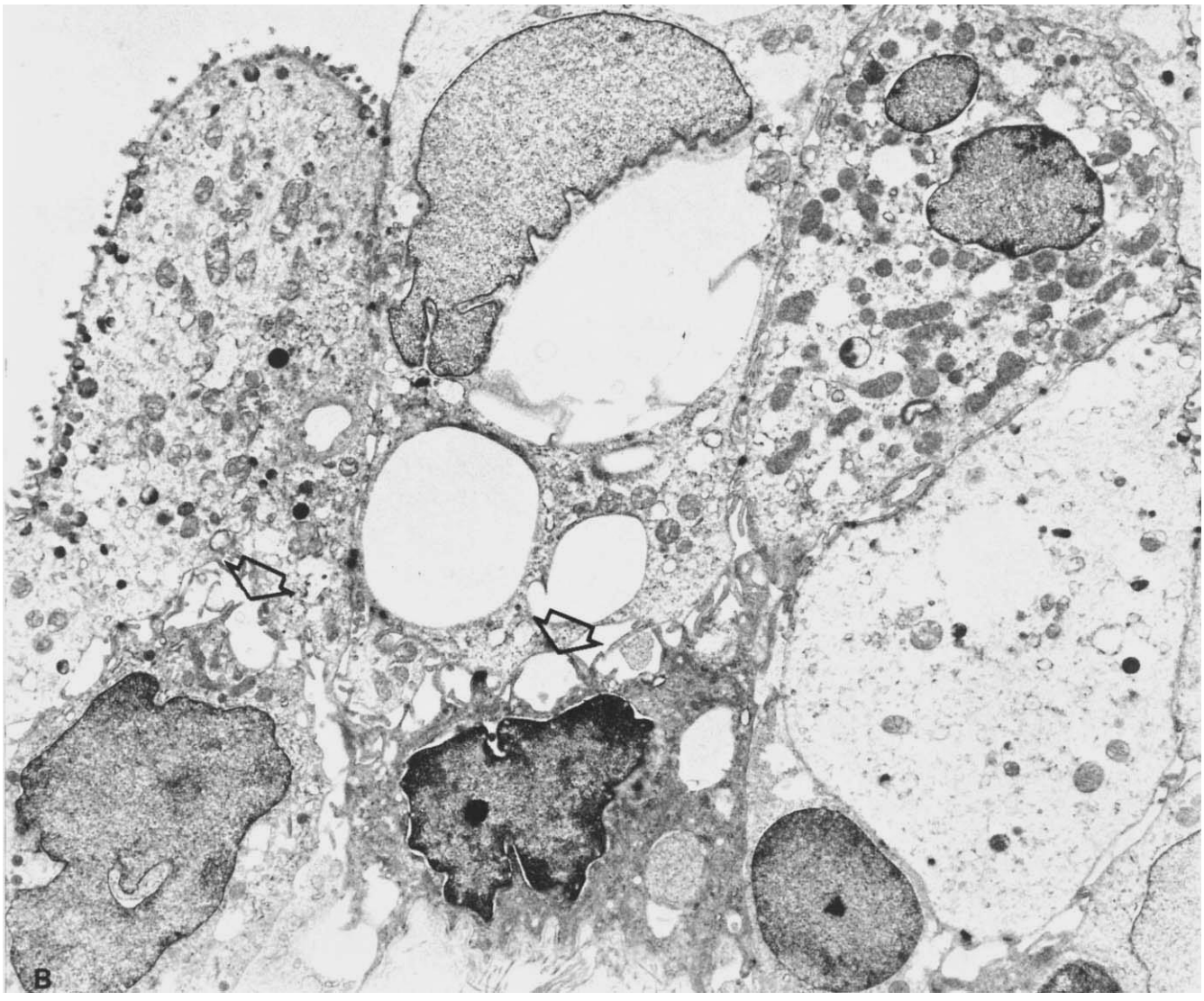


Fig. 7. *Transmission electron micrographs of toad bladder.* **A** *Incubated with 20 mU/ml of VP in the presence of an osmotic gradient without LiCl.* There is uniform swelling of the granular cells (**G**) and dilatation of the intercellular spaces (**IS**). Figure 7 continues on the following pages. (×10,476)

Examination of tissue with TEM confirmed the initial impression gained by light microscopy that the large intraepithelial vacuoles observed in the presence of LiCl, VP, and an osmotic gradient were intracellular in character and did not represent expansion of the intercellular space (Figs. 7**A** to **C**).

The characteristic appearance of toad urinary bladder bathed in amphibian Ringer's solution in the absence of LiCl and VP is depicted by SEM in Figures 8**A** and **B**. All granular cells are covered by microplicae or ridges of uniform appearance in this control preparation. With the addition of 11 mM LiCl to the

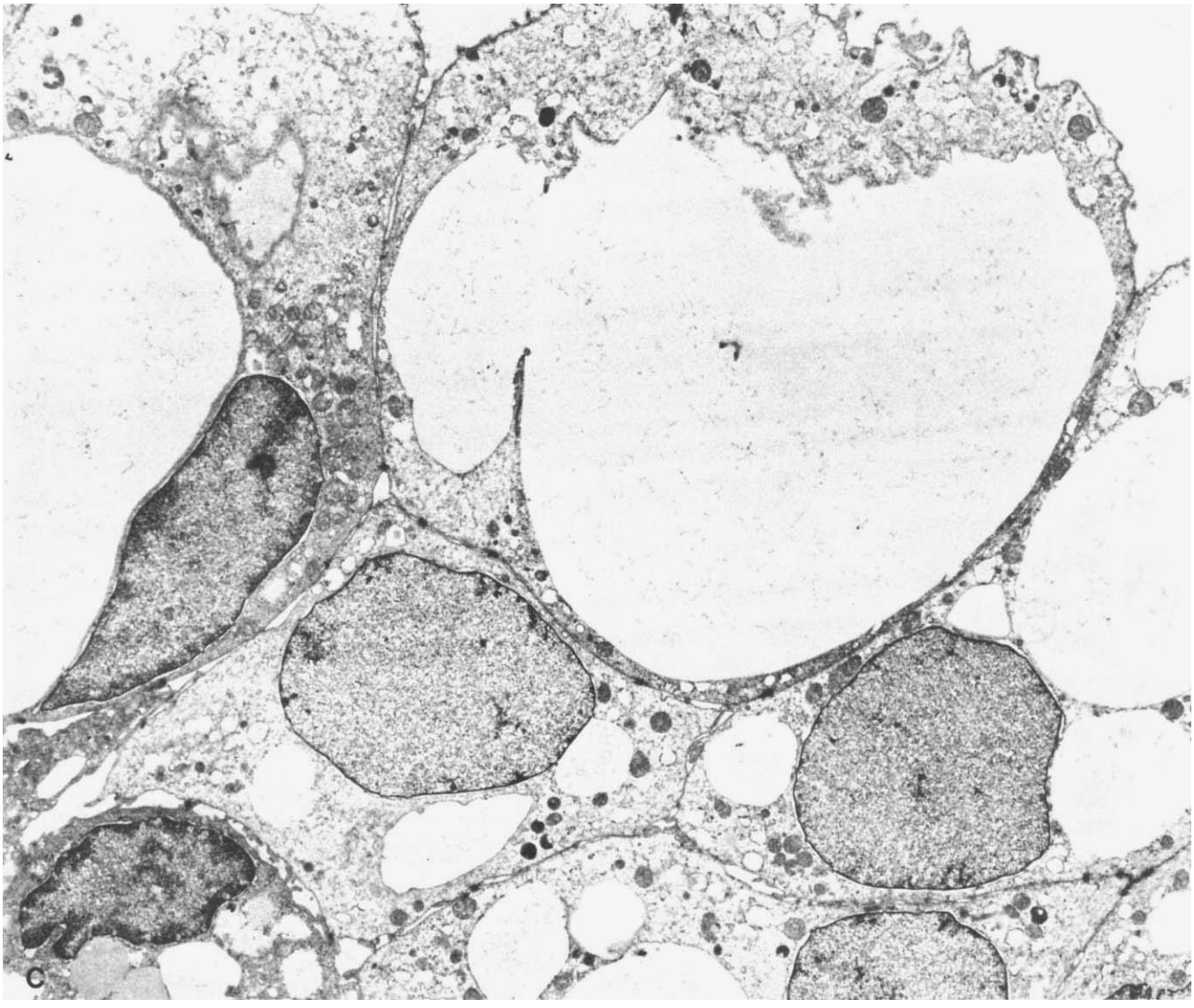


B *Incubated under similar conditions as 7A, but with mucosal solution replaced with 11 mM LiCl-Ringers.* Cell swelling (*lower right*) and intracellular vacuolization are prominent, especially in the granular cells. Intercellular spaces (*arrows*) are also dilated. (×6,887)

mucosal bathing solution, there was complete transformation of the ridges to individual microvilli in all cells in every bladder examined (Figs. 8C and D). The response to LiCl was identical to that observed following the addition of VP to the serosal bathing solution (Fig. 8E). Thus, coincident with an increase in both P_d and P_f, but with no change in P_u, there was a transformation of ridges to individual microvilli.

Discussion

This study evaluated the effects of LiCl on J_V in toad urinary bladder epithelium. Exposure of the mucosal surface to 11 mM LiCl did not inhibit VP-stimulated J_V at a hormone concentration of 20 or 100 mU/ml. However, when the concentration of VP was reduced to 1 mU/ml, mucosal exposure to LiCl significantly inhibited J_V. To minimize variability among different tissue preparations, an experimental protocol was devised to measure VP-induced J_V in the same sac preparation in both the presence and absence of LiCl. The use of this more sensitive experimental protocol might explain, at least in part, the difference between our results and those of Singer and Franko [5] who reported an inhibitory effect of 11 mM LiCl on the hydro-osmotic response induced by 100 mU/ml of VP. Although there were other differences in the experimental design such as a shorter period of LiCl exposure and the presence of a greater osmotic gradient, it seems unlikely that these differences contributed significantly to the difference in results. In addition, in the present study comparison of peak water flow rates measured at 10-min intervals instead of total water flow rates measured 30 min after VP exposure also failed to reveal an inhibitory effect of LiCl on J_V as previously suggested by the same group of investigators [5]. In our study peak J_V occurred 10 to 20 min after VP administration in all bladders independent of the presence or absence of LiCl. No significant difference in VP-induced peak J_V was detected



C *Incubated with 100 mU/ml of VP in the presence of a gradient and mucosal 11 mM LiCl-Ringers.* Cell swelling and intracellular vacuolization are especially prominent. (×5,141)

between those bladders exposed to LiCl and 20 or 100 mU/ml of VP (−19.3 ± 5.8% and −17.1 ± 5.5%, respectively) and those exposed to VP alone (−11.4 ± 6.1%; P = NS).

It is well known that the transport processes described in the toad urinary bladder resemble those occurring in the mammalian distal nephron. Therefore, it is not surprising that several in vivo studies have demonstrated that LiCl impairs the responsiveness of the collecting duct to VP in experimental animals [1, 7, 14, 15] and humans [1, 4, 16]. Hochman and Gutman [15] and Jenner and McNeil [2] have shown that LiCl antagonized the response of the rat collecting duct to VP at a wide range of hormone concentrations. However, examination of these data reveals that the inhibition by LiCl of the usual increase in urine osmolality [15] and decrease in urine volume [2, 15] that is associated with VP administration was greater in magnitude at low concentrations of the hormone. These in vivo observations compare quite favorably with our finding of an inhibitory effect of LiCl on J_V in bladders exposed to 1 mU/ml of VP, but the failure to observe a similar effect with 20 and 100 mU/ml of VP.

The demonstration by SEM that LiCl exposure of the mucosal surface of the hemibladder was associated with the transformation of microplicae to individual microvilli suggested that LiCl alone was capable of increasing the water permeability of the apical membrane of the granular cell. This hypothesis was based, in part, on the results of earlier studies from our laboratory in which VP, cAMP, and serosal hypertonicity all caused a similar transformation, the former two in both the presence and absence of an osmotic gradient [12, 17]. All three conditions increase the water permeability of the apical membrane of the granular cell. To test this hypothesis further, P_f, P_d, and P_u were measured with LiCl alone and with LiCl and VP. Values for both P_f and P_d were doubled in comparison with control hemibladders when 11 mM LiCl was substituted for NaCl in the mucosal bath, while no change was found in P_u.

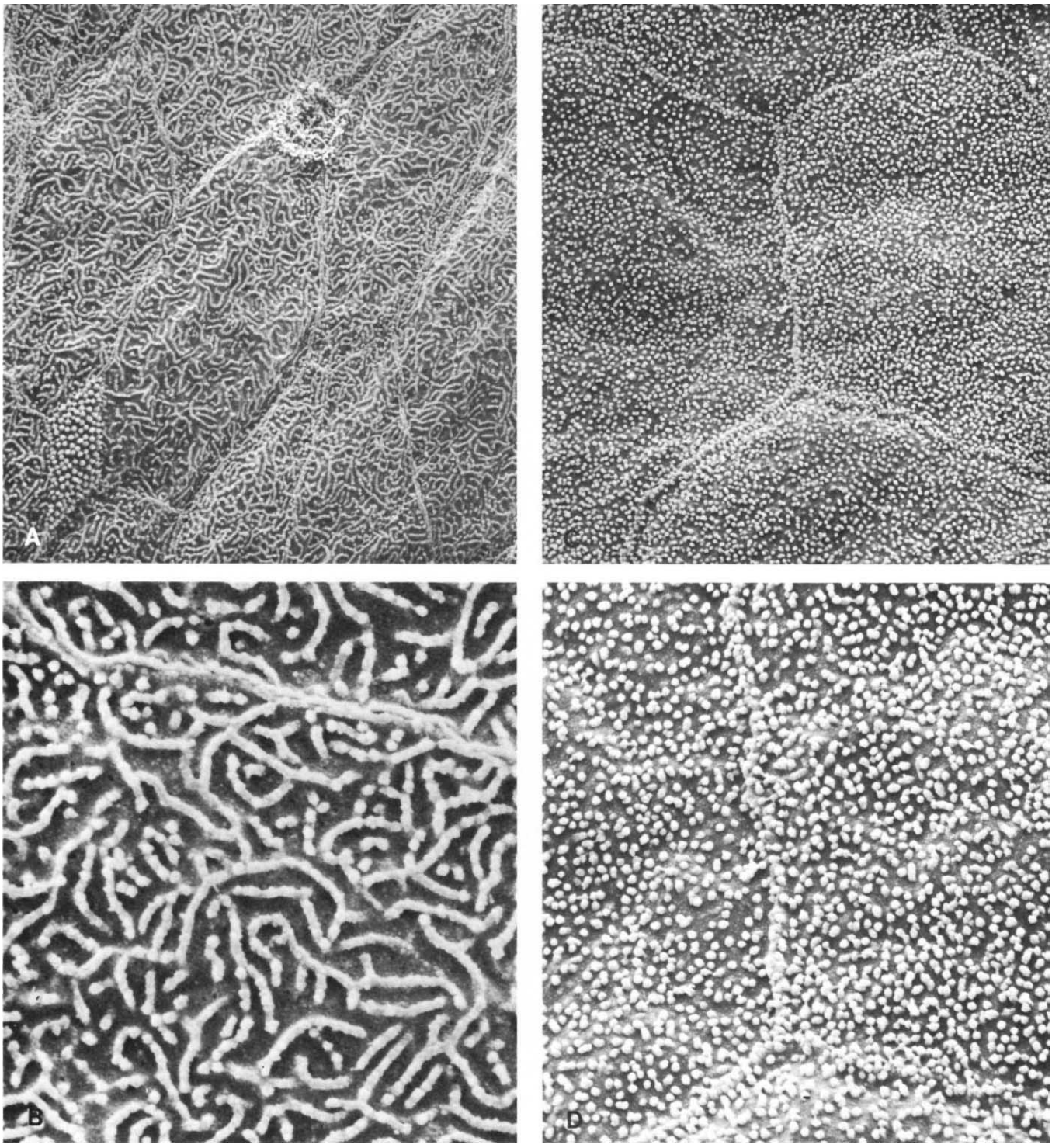


Fig. 8. *Scanning electron micrograph of toad bladder.* **A** *Bathed in Ringer's solution in the presence of an osmotic gradient (dilute mucosa).* The granular cells exhibit a uniform ridge-like surface structure. A goblet cell is depicted at the *upper right* of the picture, and a mitochondria-rich cell covered with microvilli is shown at the *lower left.* (×3,600) **B** *A higher magnification picture of the same experimental condition illustrates portions of two adjacent granular cells with the ridge-like surface structure.* (×11,250) **C** *Incubated in 11 mM LiCl-Ringer's solution in the presence of an osmotic gradient (dilute mucosa).* Portions of five granular cells are covered by individual microvilli. (×3,600) **D** *Incubated under similar conditions as tissue depicted in Figure 8C showing microvilli at a higher magnification.* Figure 8 continues on the next page. (×6,250)

Light microscopy and TEM of hemibladders exposed to LiCl alone in the presence of an osmotic gradient revealed mild cell swelling in the absence of intracellular vacuolization or expansion of the intracellular spaces (Fig. 4**B**). With the addition of 1 mU/ml of VP, cell swelling and intracellular vacuolization were prominent (Fig. 5**B**), while net J_V across the epithelium was

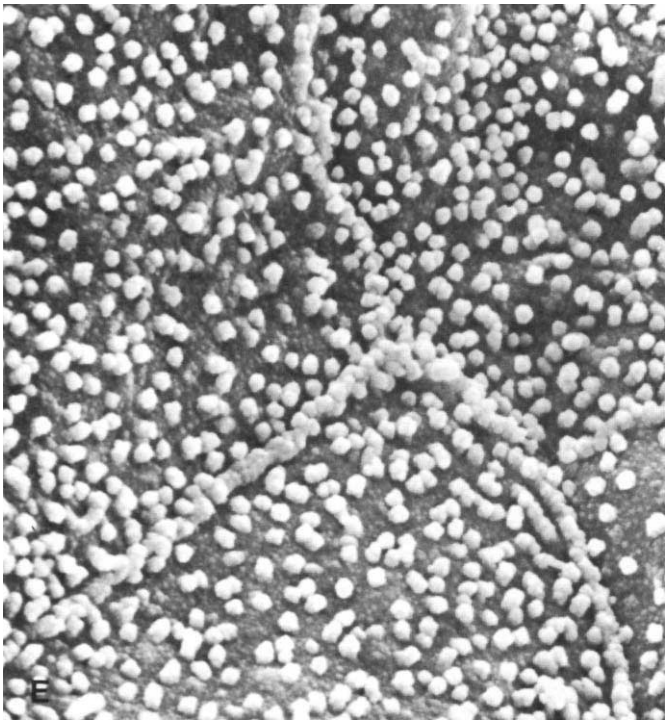


Fig. 8 E *Incubated in Ringer's solution in the presence of an osmotic gradient and 1 mU/ml of VP.* Portions of three adjacent granular cells are covered by individual microvilli identical in appearance to those observed after exposure to mucosal LiCl. (×10,900)

impaired. These are the morphological features one would anticipate if LiCl as well as VP was capable of increasing the water permeability of the apical plasmalemma, but the exit step for water removal from the cell somewhere beyond the apical cell membrane was impaired. At higher doses of VP in the presence of LiCl, intracellular vacuolization was still present, but no significant effect on transepithelial water flow was detected. The larger doses of VP were somehow able to overcome the inhibitory effects of LiCl on VP-induced transepithelial water movement. In addition, while LiCl alone resulted in a modest degree of cell swelling in the presence of an osmotic gradient, the addition of VP to the serosal bath was critical for the development of large intracellular vacuoles. Thus, while LiCl is capable of increasing both P_f and P_d, the magnitude of the permeability changes and, hence, the increase in J_V was insufficient to accentuate the altered pathway(s) of water movement across the epithelium occurring in the presence of LiCl. These findings are similar to those reported in toad urinary bladders pretreated with cytochalasin B before exposure to VP in the presence of an osmotic gradient [18]. Employing SEM in this experimental setting, LeFurgey, Dratwa, and Tisher [18] observed transformation of microplicae to microvilli, while TEM revealed the presence of large intracellular vacuoles in association with significant impairment in J_V. Similar structural and functional results were reported by Abramow and Dratwa [19] with in vitro perfusion of rabbit collecting ducts pretreated with cytochalasin B and exposed to VP in the presence of an osmotic gradient. In contrast with the present studies, however, the large dilated intracellular vacuoles appeared to contribute to the blunted response to VP in the cytochalasin B studies [18–20].

LiCl decreased sodium transport significantly as determined by measurement of SCC and the PD in both the presence and absence of VP. The sodium transport data are consistent with the earlier studies of Singer and Franko [5] who also demonstrated that the effects of lithium on SCC could be reversed by amiloride. Since the decrease in PD and SCC during basal conditions was of the same order of magnitude, no significant effect on R was observed in LiCl-treated bladders. Although VP-stimulated SCC and PD were significantly inhibited by LiCl at all hormone concentrations tested, an increase in R was observed only in the presence of LiCl and the higher VP concentrations of 20 and 100 mU/ml. The precise mechanism responsible for the inhibition of sodium transport and the natriferic response to VP by LiCl remains unclear. The findings may reflect competition between lithium and sodium ions for essential transport sites at the epithelial cell membrane because of their basic structural similarity. As a consequence sodium entry into the cell is diminished. Evidence supporting this explanation emerges from studies in frog skin [21–23] and frog [24, 25] and toad [5, 26, 27] urinary bladder that strongly suggest that lithium ions use the same transport pathway as sodium ions to gain access across the mucosal membrane. On the other hand, the reduction in sodium transport observed in the presence of lithium might be the result of a direct effect of the ion on the sodium "pump." In this regard it has been demonstrated that lithium ions are poorly transported by the sodium "pump" localized in the basolateral membrane, thus accounting for the inhibition of net sodium transport induced by lithium [28].

In summary, our findings demonstrate that mucosal LiCl enhances transepithelial water movement in toad urinary bladder epithelium as indicated by a significant increase in basal J_V, P_f, and P_d. SEM revealed that the increase in water permeability induced by LiCl was associated with transformation of the typical ridge-like surface pattern of granular cells to elongated microvilli. This morphological response is similar to that which has been noted during stimulation of transepithelial water transport with VP, cyclic-AMP, and serosal hypertonicity [17, 29, 30]. Our data also indicate that the inhibitory effect of mucosal LiCl on VP-stimulated J_V is evident only at low concentrations of the hormone. LiCl did not alter either basal or VP-stimulated P_u, thus providing additional evidence that urea and water cross the apical membrane of the granular cell via separate pathways. Finally, in agreement with previous reports [4–6] a consistent inhibition of sodium transport (SCC) by LiCl was observed regardless of the presence or absence of VP.

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