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Relationships Between Serosal Medium Potassium Concentration and Sodium Transport in Toad Urinary Bladder

II. Effects of Different Medium Potassium Concentrations on Epithelial Cell Composition

Bridget A. Robinson and Anthony D.C. Macknight

Department of Physiology, University of Otago Medical School, Dunedin, New Zealand

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Summary. Epithelial cells from hemibladders incubated in potassium-free sodium Ringer's serosal medium lost potassium, both in exchange for serosal sodium and with chloride and water. Cellular sodium of mucosal origin did not change. The loss of cellular potassium, chloride and water closely followed the fall in short-circuit current (SCC). One third as much potassium, chloride and water were lost in 1 mM potassium serosal medium; SCC fell 1/3 as much. Potassium-free choline Ringer's serosal medium abolished the initial increase in SCC and reduced the fall in cellular potassium, chloride and water and in SCC. Ouabain (10^{-2} M) in potassium-free medium prevented the initial increase in SCC and the loss of cellular chloride and water. Ouabain (5×10^{-4} M) caused loss of cellular potassium in exchange for mucosal and serosal sodium, effects different from those of absence of serosal potassium although SCC was similarly inhibited. Sodium-free mucosal medium abolished SCC and prevented the initial transient of SCC and diminished loss of cellular potassium, chloride and water on removing serosal potassium. When serosal potassium concentration was increased considerably, cells gained potassium, chloride and water, and in 116 mM potassium media, lost sodium of serosal origin. A hypothesis is advanced to explain the transients in SCC on changing serosal potassium concentration. The fall in cellular potassium, not water, probably inhibits sodium transport in media of less than 2 mM potassium.

Transepithelial sodium transport by the toad bladder depends on the presence of potassium in the serosal medium. When potassium is removed from the serosal medium, SCC and p.d. fall to low levels (Bentley, 1960; Hays & Leaf, 1961; Essig & Leaf, 1963; Davies, Martin & Sharp, 1968; Mendoza, 1972; Robinson & Macknight, 1976*a*) as does net sodium flux across the bladder (Frazier, Dempsey & Leaf, 1962; Essig & Leaf, 1963; DeGraeff, Dempsey, Lameyer & Leaf, 1965; Robinson & Macknight, 1976*a*). Removal of mucosal potassium alone had

no effect on SCC, while simultaneous removal of mucosal and serosal potassium had the same effects on SCC as removal of serosal potassium (Finn, Handler & Orloff, 1967; Mendoza, 1972; Robinson & Macknight, 1976*a*).

At least two explanations have been advanced to explain this inhibition of SCC on removal of serosal potassium. Firstly, using the Koefoed-Johnsen and Ussing (1958) model for transepithelial sodium transport, there would be no potassium at the serosal membranes of the cells to be taken up in one-for-one exchange with sodium extruded from the cells. This hypothesis is not supported by all experiments (Essig & Leaf, 1963; Robinson & Macknight, 1976*a*), nor is it likely that potassium uptake is rigidly coupled to sodium extrusion in either toad urinary bladder (Essig, Frazier & Leaf, 1963; Frazier & Leaf, 1963; Finn, 1974; Macknight, Civan & Leaf, 1975*a*; Robinson & Macknight, 1976*b*) or frog skin (Cereijido & Curran, 1965; Biber, Aceves & Mandel, 1972).

An alternative hypothesis, proposed by Essig and Leaf (1963) from the results of analysis of whole hemibladders, was compatible with electrogenic sodium extrusion and attributed inhibition of SCC in the absence of serosal potassium to inhibition of sodium entry to the epithelial cells through their mucosal membranes. The effect was thought to be mediated by the loss in tissue potassium and gain in tissue sodium that occurred. However, the decrease in whole tissue sodium transport pool recorded by Essig and Leaf (1963), upon which their hypothesis was based, may have merely reflected the decrease in net sodium flux across the tissue, with less ^{24}Na being in transit through the tissue after sodium transport was inhibited. Results of analysis of whole hemibladders are not reliable, for changes in nonepithelial components would tend to predominate over changes in the epithelial cells themselves, which make up less than 20% of the total composition of whole hemibladders (Macknight *et al.*, 1975*a*). Since Finn, Handler and Orloff (1966) detected a decrease in whole tissue chloride and water, besides the gain in sodium and loss of potassium, after prolonged incubation in potassium-free media, and experiments with frog skin demonstrated cellular shrinkage in potassium-free media (Ussing, 1965), it is possible that the effects of removal of serosal potassium are mediated by cellular shrinkage.

Therefore, the effects of absence of serosal potassium on the composition and sodium transport pool of the epithelial cells themselves were determined in an attempt to elucidate how removal of serosal potassium inhibits sodium transport. Since nothing is yet known of the effects

of serosal media of high potassium concentration on epithelial cell composition and sodium transport pool in toad urinary bladder, these were also studied.

Materials and Methods

The methods used in these experiments have been presented in detail elsewhere (Macknight, DiBona, Leaf & Civan, 1971*a*; Macknight *et al.*, 1975*a*; Robinson & Macknight, 1976*a*) and are summarized below. The compositions of the media used are shown in Table 1 of the preceding paper (Robinson & Macknight, 1976*a*). In addition, a hypotonic K-free Na Ringer's was used which differed from K-free Na Ringer's in having only 77 mM and 79 mM Cl, and an osmolarity of 150–160 mosm/liter.

Ouabain octahydrate (Sigma Chemical Company, St. Louis) and amiloride (the gift of Merck, Sharp and Dohme, NZ, Ltd.) were used in some media. Inulin-carboxyl-^{14}C and inulin-methoxy-^{3}H were obtained from New England Nuclear Corporation; ^{24}Na was obtained from Australian Atomic Energy Commission, Lucas Heights, NSW.

Hemibladders from female Dominican toads of the species *Bufo marinus* were mounted in chambers and bathed with Na Ringer's. Hemibladders were continuously short-circuited. Once SCC had stabilized, the chambers were drained and refilled with media of compositions indicated in Results.

When the serosal chamber was to be filled with K-free Na Ringer's, it was washed through five times with K-free Na Ringer's, and then filled with K-free Na Ringer's. The serosal medium was drained and washed out with fresh K-free Na Ringer's after 15 and 30 min. These washing procedures kept the serosal medium potassium concentration, determined by flame photometry, at less than 0.2 mM throughout all experiments.

In some experiments, hemibladders were incubated in aerated media in beakers rather than in chambers. However, the experimental procedure was exactly analogous to that of experiments using chambers.

After incubation, the chambers were drained and the hemibladders were cut from them (or hemibladders were taken from the beakers), blotted and the epithelial cells scraped off using a glass slide, as described by Macknight *et al.*, 1971*a*. The epithelial cells were transferred to Pyrex tubes, their wet weight determined, and then were counted for ^{24}Na in a gamma scintillation spectrometer (Packard Instrument Company, Inc.) together with samples of the media.

Tissue water content was taken to be equal to the loss of weight when the epithelial cell scrapings were dried in a hot air oven at 105 °C. The tissue was extracted in 10 ml of 0.1 M nitric acid for 7–9 days, sufficient time for ^{24}Na to decay. Sodium and potassium contents of the extract were determined by flame photometry, and chloride by potentiometric titration. Samples of the extract and bathing media were counted for ^{14}C and ^{3}H in a liquid scintillation spectrometer (Packard Instrument Company, Inc.) as described by Macknight *et al.* (1975*a*).

Cellular water and ion contents were corrected for contamination with extracellular fluid by assuming that isotopically labelled inulin equilibrated in the extracellular space and that the ions in this space were at the same concentration as in the bulk medium. Corrections for contamination by the mucosal and serosal media could be made separately, by the use of ^{14}C-inulin in the mucosal medium and ^{3}H-inulin in the serosal medium. McIver and Macknight (1974) have established that inulin is a suitable extracellular marker for toad bladder epithelial cells under these conditions. Cellular sodium content determined by ^{24}Na was calculated as described by Macknight *et al.* (1975*a*).

Cellular water is expressed as kg water per kg dry weight of epithelial cell scrapings and cellular ions as mmole per kg dry weight or mmole per kg cellular water. Most

experiments were performed using one hemibladder of each pair as the control for the other. Means of paired analyses are presented with their difference and standard error. Significances of differences between means have been evaluated using Student's *t*-test. Results of unpaired analyses are presented as mean ± SEM.

Results

Experiments described in a subsequent paper (Robinson & Macknight, 1976*b*) established that the exchange of potassium across the mucosal cell membranes was negligible – less than 1.5% of cellular potassium was labelled by mucosal ^{42}K after 60 min ($n=7$). In addition, the effects of removing all potassium from both the mucosal and serosal media were identical to those of removing just serosal potassium (Robinson & Macknight, 1976*a*). Therefore incubating hemibladders in beakers in K-free Na Ringer's as was done in some experiments should be comparable to incubation in chambers with potassium absent from only the serosal medium.

(*i*) *Effects of Potassium-free Sodium Ringer's Serosal Medium on the Composition of Epithelial Cells*

The composition of epithelial cells scraped from hemibladders incubated in K-free Na Ringer's serosal medium for 60 min was compared with that of cells from hemibladders incubated in Na Ringer's for 60 min. The mucosal medium was Na Ringer's containing tracer amounts of ^{24}Na. The results are presented in Table 1.

Cells from hemibladders exposed to a K-free Na Ringer's serosal medium had lost potassium, chloride and water and gained sodium when compared with controls. There was no significant difference in ^{24}Na content. Cellular potassium and chloride concentrations were decreased, sodium concentration increased and ^{24}Na concentration unchanged after removal of serosal potassium. About 50 mmole/kg dry wt of potassium appeared to have been lost in exchange for 50 mmole/kg dry wt of sodium gained. The remaining 60 mmole/kg dry wt of potassium had been lost with 60 mmole/kg dry wt of chloride and an approximately isosmolal amount of water. Altogether the cells lost about one-third of their total potassium over 60 min. The sodium gained by the epithelial cells must have come from the serosal solution, for there was no corresponding increase in ^{24}Na content.

Table 1. Effect of potassium-free serosal medium on epithelial cell composition

Serosal solution	Cell water (kg/kg dry wt)	Cell ions (mmole/kg dry wt)			
		Na	^{24}Na	K	Cl
Na Ringer's	3.14	200	43	421	208
K-free Na Ringer's	2.70	247	38	305	145
$\Delta \pm$ SE	-0.44 ± 0.07	47 ± 11	-5 ± 4	-116 ± 8	-63 ± 11
p	<0.001	<0.005	>0.10	<0.001	<0.001
$n=12$					
		Cellular ion concentration (mmole/kg IC H_2O)			
		Na	^{24}Na	K	Cl
Na Ringer's		64	14.1	135	68
K-free Na Ringer's	K-free Na	95	14.6	116	57
$\Delta \pm$ SE		30 ± 5	0.6 ± 1.1	-19 ± 3	-11 ± 4
p		<0.001	>0.60	<0.001	<0.02
$n=12$					

Paired hemibladders were mounted in Ussing-type chambers and continuously short-circuited. After an initial incubation in Na Ringer's solution, mucosal and serosal solutions were replaced with fresh Na Ringer's solutions; the mucosal solution included ^{14}C-inulin and ^{24}Na. After 40 min of incubation the serosal medium of one hemibladder was drained, and washed through 5 times with K-free Na Ringer's before being filled with K-free Na Ringer's with ^{3}H-inulin. The serosal side of the control hemibladder of each pair was washed with Na Ringer's, and filled with Na Ringer's with ^{3}H-inulin. Serosal solutions were replaced after 15 and 30 min. After 60 min the bladders were taken from the chambers, blotted and the epithelial cells scraped off for analysis.

Therefore, removal of serosal potassium resulted in an exchange of serosal sodium for cellular potassium, and a loss of cellular potassium with chloride and water. No detectable change in ^{24}Na content occurred. For the results shown in Table 1, a change in ^{24}Na content of greater than 8 mmole/kg dry wt would have been significant assuming the SE of ± 3.5 mmole/kg dry wt in the experiments. Thus a 20% change in cellular mucosal sodium pool would have been detectable. The techniques used are sensitive enough to show considerable changes in cellular ^{24}Na content after exposure of hemibladders to amiloride and to ouabain (Macknight *et al.*, 1975*a, b*) and to antidiuretic hormone (Macknight, Leaf & Civan, 1970, 1971*b*).

Experiments were performed to examine the time course of the changes in epithelial cellular composition when exposed to K-free Na Ringer's serosal medium (Fig. 1). Results are taken from several experiments, some performed with paired hemibladders mounted in Ussing-type cham-

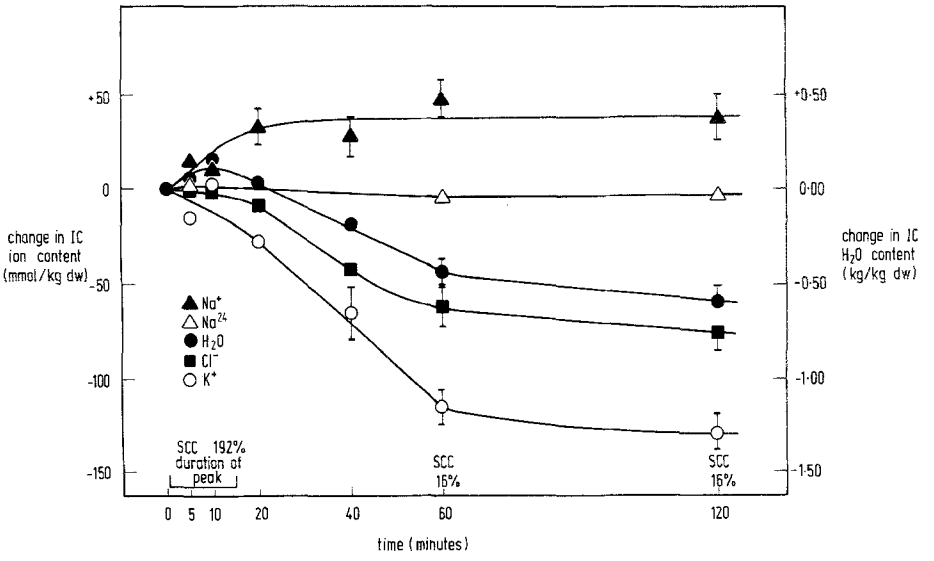

Fig. 1. Change in intracellular composition, with time, in potassium-free media. Results are pooled from a number of experiments using the same batch of toads. Five, 60 and 120-min values come from experiments performed with chambers. The 60-min values are taken from Table 1. Ten, 20 and 40-min values are taken from experiments where hemibladders were incubated in beakers. The graph shows the mean difference in cellular composition from controls in Na Ringer's. Bars indicate SE where this difference is significant ($p < 0.05$). $n = 8$ or 12

bers, and others performed with hemibladders incubated in beakers. Hemibladders incubated in beakers are in effect short-circuited by the medium.

Fig. 1 shows the large loss of potassium by the cells, balanced partly by a gain of cellular sodium and partly by a loss of cellular chloride with water. The exchange of cellular potassium for serosal sodium was complete within the first 20 min of incubation in the potassium-free medium, while the loss of potassium with chloride and water though only apparent after the first 10–20 min continued until about 60 min.

Cellular ^{24}Na content was not significantly different from control values after 5, 60 or 120 min in K-free Na Ringer's serosal medium. If cellular ^{24}Na content were decreased by removal of serosal potassium, ^{24}Na content should have been lowest in those hemibladders which showed the greatest inhibition of SCC in K-free Na Ringer's. However, there was no correlation at all between the ^{24}Na content of the epithelial cells and the final SCC after 60 min in K-free Na Ringer's serosal medium (for 55 hemibladders, correlation coefficient was 0.0934, $p > 0.40$).

Table 2. Epithelial cell composition of short-circuited and open-circuited hemibladders in the presence and absence of serosal potassium

	Cell water (kg/kg dry wt)	Cell ions (mmole/kg dry wt)			
		Na	^{24}Na	K	Cl
(*a*) Serosal medium, Na Ringer's:					
Short-circuited	3.14	187	34	425	192
Open-circuited	3.23	175	31	429	178
$\Delta \pm$ SE	0.07 ± 0.11	−12 ± 16	−3 ± 3	4 ± 16	−16 ± 10
p	>0.40	>0.40	>0.20	>0.70	>0.10
$n=8$					
(*b*) Serosal medium, K-free Na Ringer's:					
Short-circuited	2.89	254	32	305	212
Open-circuited	2.84	261	32	301	188
$\Delta \pm$ SE	−0.05 ± 0.20	7 ± 23	0 ± 2	−4 ± 19	−24 ± 26
p	>0.80	>0.70	>0.70	>0.80	>0.40
$n=4$					

One of each pair of hemibladders was continuously short-circuited while the other remained open-circuited. The hemibladders were equilibrated with Na Ringer's, with ^{24}Na and ^{14}C-inulin in the mucosal medium, for 40 min. Paired hemibladders were then incubated in Na Ringer's + ^{3}H-inulin serosal medium (*a*) or in K-free Na Ringer + ^{3}H-inulin serosal medium (*b*). After 60 min, the epithelial cells were collected for analysis.

There were no significant changes in cellular composition between 60- and 120-min incubation in K-free Na Ringer's serosal media. Thus the changes in cellular composition caused by removal of serosal potassium were largely completed within the first 60 min.

The losses of cellular potassium, chloride and water on removal of serosal potassium follow a similar time course to the inhibition of SCC (Robinson & Macknight, 1976*a*). Indeed the final SCC (as a percentage of the SCC in Na Ringer's) showed a good correlation with final cellular potassium content ($r=0.46$, $p<0.001$, $n=53$), and with final cellular water content ($r=0.45$, $p<0.001$, $n=53$). Thus SCC seemed closely related to cellular potassium and water content.

All experiments described in this paper were performed using short-circuited hemibladders. Therefore it was necessary to establish the effects of short-circuiting hemibladders on the composition of their epithelial cells (Table 2). Although the average M→S Na flux of 54 μA/mg dry wt ($n=8$) under short-circuited conditions was almost twice that of 28 μA/mg dry wt under open-circuited conditions, epithelial cellular composition was the same under the two conditions (Table 2*a*). Removal of serosal

potassium had the same effects on epithelial cell composition whether hemibladders were short-circuited or open-circuited (Table 2*b*).

(*ii*) *Effects of Hypotonic K-free Na Ringer's Serosal Medium on Cellular Composition and on SCC*

Cells from hemibladders incubated in potassium-free serosal media had lost considerable amounts of potassium and chloride and had shrunk. Since there is evidence that cellular shrinkage may inhibit transepithelial sodium transport (Ussing, 1965; Lipton, 1972), prevention of cellular shrinkage might arrest inhibition of SCC in K-free Na Ringer's serosal medium, if such inhibition were the result of loss of cellular water. Hemibladders equilibrated with Na Ringer's were bathed with K-free Na Ringer's or hypotonic K-free Na Ringer's serosal media for 60 min. Their epithelial cell compositions were determined and are shown in Table 3.

Incubation in hypotonic potassium-free serosal medium did not prevent depression of SCC in the absence of serosal potassium, although the cells contained considerably more water than paired tissue from K-free Na Ringer's. Cellular potassium content was similar in the two media, but cellular potassium concentration was less in the hypotonic medium as were cellular sodium and chloride contents. (In these experiments, cellular water, potassium and chloride in the controls were higher than usual. The reasons for these differences in cellular composition

Table 3. Effect of hypotonic K-free Na Ringer's serosal medium on epithelial cell composition and on SCC

Serosal solution	Cell water (kg/kg dry wt)	Cell ions (mmole/kg dry wt)			Final SCC %
		Na	K	Cl	SCC in NaR
K-free NaR	4.28	329	363	383	19%
Hypotonic K-free NaR	5.55	285	358	301	22%
$\Delta \pm$ SE	1.27 ± 0.15	-44 ± 11	-5 ± 12	-82 ± 14	$3 \pm 2\%$
p	<0.001	<0.005	>0.60	<0.001	>0.20

Paired hemibladders, mounted in chambers and continuously short-circuited, were equilibrated with Na Ringer's + ^{14}C-inulin, mucosa, and Na Ringer's, serosa. After 40 min, SCC was recorded. The serosal solutions were changed to either hypotonic K-free Na Ringer's + ^{3}H-inulin or K-free Na Ringer's + ^{3}H-inulin. After 60 min, epithelial cells were collected for analysis.

between batches of toads are unclear although they may represent seasonal variation. In addition, these hemibladders behaved unusually in having very high SCC's initially, which steadily fell during incubation in Na Ringer's to reach quite low steady values.)

Therefore, the change in cellular volume itself does not seem to be the cause of the inhibition of sodium transport produced by removal of serosal medium potassium. Furthermore, removal of serosal medium potassium prevented the stimulation of sodium transport which has been reported in hypotonic serosal media (Lipton, 1972).

(*iii*) *Effects of 1 mM K serosal Medium on Cellular Composition*

Epithelial cells were analyzed from hemibladders incubated 60 min in 1 mM K Na Ringer's serosal medium and compared with control cells from Na Ringer's. The mucosal medium was Na Ringer's containing ^{24}Na. The results are shown in Table 4.

Cellular potassium, chloride and water were lost, as in K-free Na Ringer's serosal medium, but in smaller amounts. There were no significant changes in cellular sodium and ^{24}Na. The SCC fell to a steady level 70% of its previous level in Na Ringer's. Thus the losses of potassium, chloride and water and the fall in SCC were all only about one-third of those in K-free Na Ringer's.

Table 4. Effects of 1 mM K-Na Ringer's serosal medium on epithelial cell composition and on SCC

Serosal medium	Cell water (kg/kg dry wt)	Cell ions (mmole/kg dry wt)			
		Na	^{24}Na	K	Cl
Na Ringer's	3.71	209	49	454	256
1 mM K-Na Ringer's	3.42	192	41	434	221
$\Delta \pm$ SE	-0.29 ± 0.13	-17 ± 17	-8 ± 3	-20 ± 5	-35 ± 12
p	>0.05	>0.30	>0.50	<0.01	<0.02
$n=8$					

Measured medium [K] = 1.1 mM.
SCC after 60 min in 1.1 mM K NaR was $70 \pm 3\%$ of SCC in NaR.
($n=8$.)
Paired hemibladders, mounted in chambers and continuously short-circuited, were incubated initially in Na Ringer's + ^{24}Na + ^{14}C-inulin mucosal medium, Na Ringer's serosal medium for 40 min. The serosal chambers were drained and filled with either 1 mM K-Na Ringer's + ^{3}H-inulin or Na Ringer's + ^{3}H-inulin. After 60 min, hemibladders were taken from the chambers, blotted and the epithelial cells scraped off for analysis.

(*iv*) *Effects of K-free Choline Ringer's Serosal Medium on Epithelial Cellular Composition*

Essig and Leaf (1963) found that SCC did not decrease as much in K-free choline Ringer's as it did in K-free Na Ringer's. This difference was associated with a smaller change in potassium and sodium in whole hemibladders. But it is not known what effects K-free choline Ringer's has on epithelial cell composition. Hemibladders were therefore incubated in K-free choline Ringer's or K-free Na Ringer's serosal media for 60 min. Then the epithelial cells were scraped off and analyzed (Fig. 2).

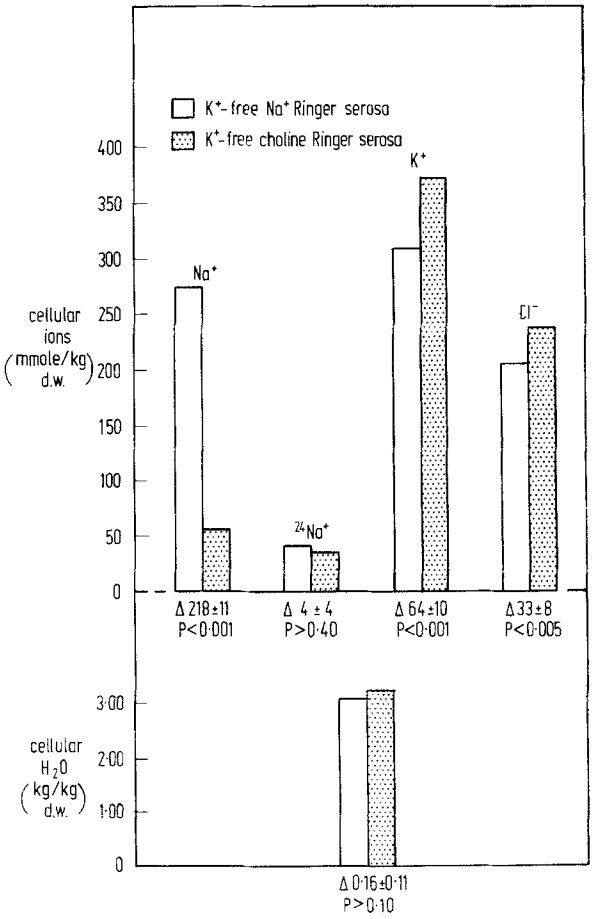

Fig. 2. Effects of serosal K-free sodium Ringer's or K-free choline Ringer's on toad bladder epithelial cell composition. Paired hemibladders, mounted in chambers and continuously short-circuited, were equilibrated with Na Ringer's + ^{24}Na + ^{14}C-inulin mucosal solution and either Na Ringer's or choline Ringer's serosal solution. After 40 min these serosal media were drained and replaced by K-free Na Ringer's + ^{3}H-inulin and K-free choline Ringer's + ^{3}H-inulin, respectively. After 60 min the epithelial cells were collected for analysis. (Mean differences ± SE are indicated; $n=7$)

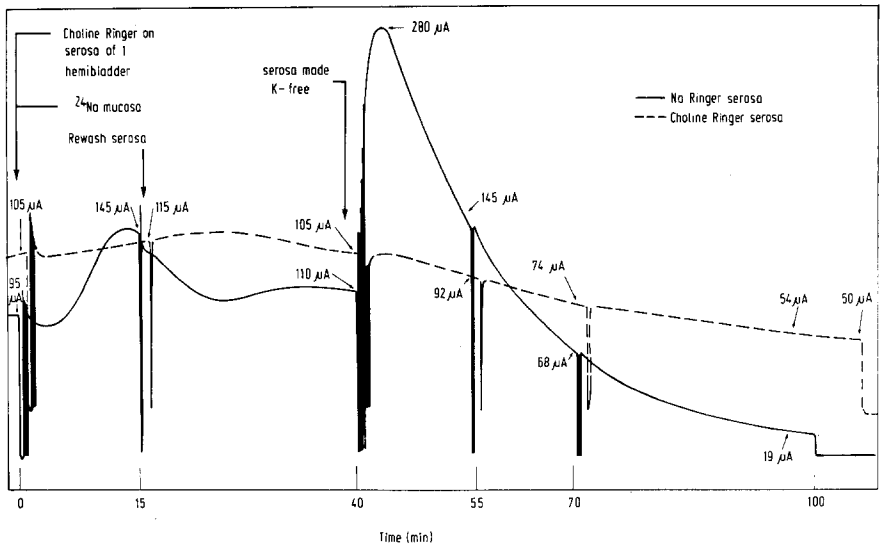

Fig. 3. Effect of a K-free choline Ringer's serosal solution compared with K-free Na Ringer's. SCC tracing from one pair of hemibladders described in Fig. 2

Cells exposed to serosal choline Ringer's lose all sodium other than that labelled by mucosal ^{24}Na (Macknight *et al.*, 1975*a*); this sodium lost must be replaced by choline from the serosal solution for cellular chloride and water contents are unaltered. Thus the differences in sodium content in the present experiments were those associated with absence of serosal sodium.

Cells exposed to K-free choline Ringer's had lost less potassium, chloride and water than cells in K-free Na Ringer's. Indeed, comparison with Table 1 indicates that cells in K-free choline Ringer's lost about one-half as much potassium, chloride and water as did cells in K-free Na Ringer's.

There was no peak of SCC when cells equilibrated with serosal choline Ringer's were then exposed to K-free choline Ringer's (Fig. 3). The SCC was $87 \pm 4\%$ ($n=8$) of its value in Na Ringer's after 40-min equilibration in choline Ringer's, and fell steadily in K-free choline Ringer's to be $58 \pm 4\%$ ($n=8$) of this value after 60 min.[1]

[1] When Na Ringer's bathes the mucosa and choline Ringer's the serosa, there are considerable gradients for ion diffusion across the hemibladder. Sodium would tend to diffuse M→S and choline to diffuse S→M. But transepithelial sodium permeability, determined from S→M ^{24}Na flux measurements, is very low, as is choline permeability (Leaf & Hays, 1962). Therefore, SCC is not likely to deviate much from net sodium flux. In addition, SCC still agreed closely with net M→S Na flux under these conditions (Frazier *et al.*, 1962) and net sodium movement seemed to represent active transport of sodium (Essig, 1965).

These experiments support the relation between SCC and cellular composition indicated in the previous experiments, for a smaller depression of SCC was associated with smaller losses of potassium, chloride and water.

(*v*) *Effects on Cellular Composition of Ouabain in the Presence and Absence of Serosal Potassium*

Ouabain specifically inhibits Na-K-linked ATPase in many tissues (Skou, 1965). It causes a rapid decrease in transepithelial SCC when added to the serosal medium bathing toad bladders. Supramaximal inhibitory concentrations of ouabain (10^{-2} M) caused a loss of cellular potassium, a gain in sodium of mucosal and serosal origin, but no change in cellular chloride or water contents (Macknight *et al.*, 1975*b*). Thus, the effects of ouabain appear different from those of removal of serosal potassium (Table 1), and experiments were performed to confirm this.

Epithelial cell composition was determined for hemibladders equilibrated for 60 min with either 10^{-2} M ouabain or K-free Na Ringer's serosal medium (Table 5). Cells from K-free Na Ringer's had less water, sodium, ^{24}Na and chloride than cells exposed to ouabain. Although ouabain caused a similar loss of potassium to K-free Na Ringer's, it was balanced entirely by a gain of sodium from the mucosal and serosal media, so that cellular chloride and water did not change. The SCC

Table 5. Effects of ouabain, 10^{-2} M, and of absence of serosal K on cellular composition

Serosal solution	Cell water (kg/kg dry wt)	Cell ions (mmole/kg dry wt)			
		Na	^{24}Na	K	Cl
NaR + ouabain	3.33	319	125	286	217
K-free NaR	2.74	268	26	266	167
$\Delta \pm$ SE	-0.59 ± 0.10	-51 ± 29	-98 ± 19	-20 ± 16	-50 ± 17
p	<0.001	>0.10	<0.005	>0.20	<0.025
$n=7$					

Hemibladders, in chambers and short-circuited, were initially incubated in Na Ringer's. After 40 min with ^{24}Na in the mucosal medium, the serosal Na Ringer's was drained. One hemibladder of each pair was then bathed with Na Ringer's + 10^{-2} M ouabain, while the other was bathed with K-free Na Ringer's. Both serosal media contained ^{3}H-inulin, and the mucosal solution contained ^{14}C-inulin. The bladders were taken from the chambers after 60 min and the epithelial cells analyzed.

Table 6. Effect of ouabain, 5×10^{-4} M, on epithelial cell composition and on SCC

Serosal solution	Cell water (kg/kg dry wt)	Cell ions (mmole/kg dry wt)			
		Na	^{24}Na	K	Cl
NaR	4.08	268	56	421	350
NaR + ouabain	4.21	358	104	326	362
$\Delta \pm$ SE	0.13 ± 0.10	90 ± 18	48 ± 18	−95 ± 9	12 ± 10
p	> 0.20	< 0.001	< 0.05	< 0.001	> 0.20
$n=8$					

SCC after 60 min with 5×10^{-4} M ouabain, serosa: 21 ± 4% of previous SCC in Na Ringer's.

Paired hemibladders mounted in chambers were short-circuited and incubated in Na Ringer's. The mucosal solution included ^{24}Na and ^{14}C-inulin. Forty minutes later, the serosal solutions were drained. One hemibladder received a Na Ringer's + 5×10^{-4} M ouabain serosal solution; the paired hemibladder Na Ringer's. Both serosal media contained ^{3}H-inulin. After 60 min, the solutions were drained, the hemibladders taken down, blotted, and the epithelial cells scraped off for analysis.

fell to 1 ± 1% ($n=24$) after a 60-min incubation with 10^{-2} M ouabain—thus, SCC was inhibited more than by the absence of serosal potassium.

Therefore 5×10^{-4} M ouabain was employed to inhibit SCC as much as did K-free Na Ringer's serosal medium over 60 min. Cellular potassium was lost, and sodium gained from the mucosal and serosal media (Table 6). There was no change in cellular chloride and water contents. Therefore, even when ouabain inhibited SCC to a similar extent as K-free Na Ringer's serosal medium, the effects of ouabain on cellular composition were quite unlike those due to removal of serosal potassium.

The effects of 10^{-2} M ouabain on hemibladders bathed with K-free Na Ringer's were then studied (Table 7). When ouabain was present, more potassium but less chloride and water were lost and more sodium was gained, due to uptake of mucosal sodium, than in K-free Na Ringer's serosal medium alone (Table 7*b*). When ouabain was present, cells bathed in K-free Na Ringer's serosal medium still gained serosal sodium in exchange for potassium, but there was an additional gain of sodium from the mucosal medium in exchange for cellular potassium. Ouabain had the same effect in the absence of serosal potassium as in its presence (Table 7*c*); therefore ouabain prevented the losses of chloride and water that usually occurred on removal of serosal potassium. When ouabain was present in the absence of serosal potassium, the effect of ouabain predominated over that of removal of serosal potassium.

Table 7. The effects of ouabain, 10^{-2} M in the presence and absence of serosal K, on composition of toad bladder epithelial cells

Serosal solution	Cell water (kg/kg dry wt)	Cell ions (mmole/kg dry wt)			
		Na	^{24}Na	K	Cl
(*a*) NaR	3.10	191	45	408	217
K-free NaR	2.58	255	42	284	170
$\Delta \pm$ SE	-0.52 ± 0.08	63 ± 10	-3 ± 5	-124 ± 8	-47 ± 5
p	<0.001	<0.001	>0.5	<0.001	<0.001
$n=8$					
(*b*) K-free NaR	2.57	245	34	292	167
K-free NaR + ouabain	2.84	323	117	254	208
$\Delta \pm$ SE	0.27 ± 0.07	77 ± 11	83 ± 13	-38 ± 5	41 ± 5
p	<0.005	<0.001	<0.001	<0.001	<0.001
$n=8$					
(*c*) K-free NaR + ouabain	3.37	365	113	255	243
NaR + ouabain	3.33	379	107	278	257
$\Delta \pm$ SE	-0.04 ± 0.12	14 ± 18	-6 ± 6	23 ± 14	14 ± 14
p	>0.7	>0.4	>0.3	>0.1	>0.3
$n=8$					

Three series of experiments with paired hemibladders were performed. After short-circuited hemibladders, in chambers, had equilibrated 40 min with Na Ringer's $+^{24}$Na $+^{14}$C-inulin mucosal medium and Na Ringer's serosal medium, the serosal chambers were drained. The serosal chambers were filled with either K-free Na Ringer's, K-free Na Ringer's $+10^{-2}$ M ouabain, Na Ringer's $+10^{-2}$ M ouabain or Na Ringer's. All these media contained ^{3}H-inulin. Sixty minutes later, hemibladders were taken from the chambers, blotted and the epithelial cells scraped off for analysis. [Experiments in (*c*) were performed two months prior to those in (*a*) and (*b*).]

Ouabain abolished the initial transient increase in SCC when added to the K-free Na Ringer's serosal medium after the fifth washing of the serosa (Fig. 4). Ouabain caused an initial very rapid decline in SCC followed by a slow, steady decrease to $5 \pm 2\%$ ($n=28$) of the SCC in Na Ringer's over 60 min. This also occurred when serosal potassium was present (Fig. 5); the rate of the initial decline in SCC was identical in the presence or absence of potassium ($n=8$). The immediate decrease in SCC after ouabain presumably reflects the rapidity with which ouabain diffused to and interacted with the transport sites on the serosal membrane of the epithelial cells.

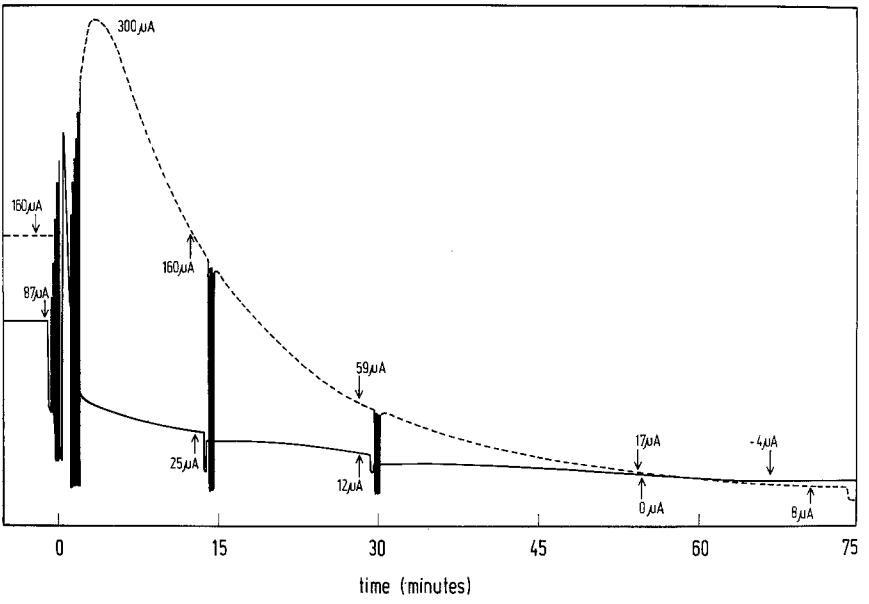

Fig. 4. Effect of potassium-free serosal medium, with or without 10^{-2} M ouabain, on the short-circuit current of paired hemibladders. Record of SCC for a typical pair of hemibladders in experiment (*b*) of Table 7. Solid line = K-free Na Ringer's + 10^{-2} M ouabain, serosa; dashed line = K-free Na Ringer's, serosa

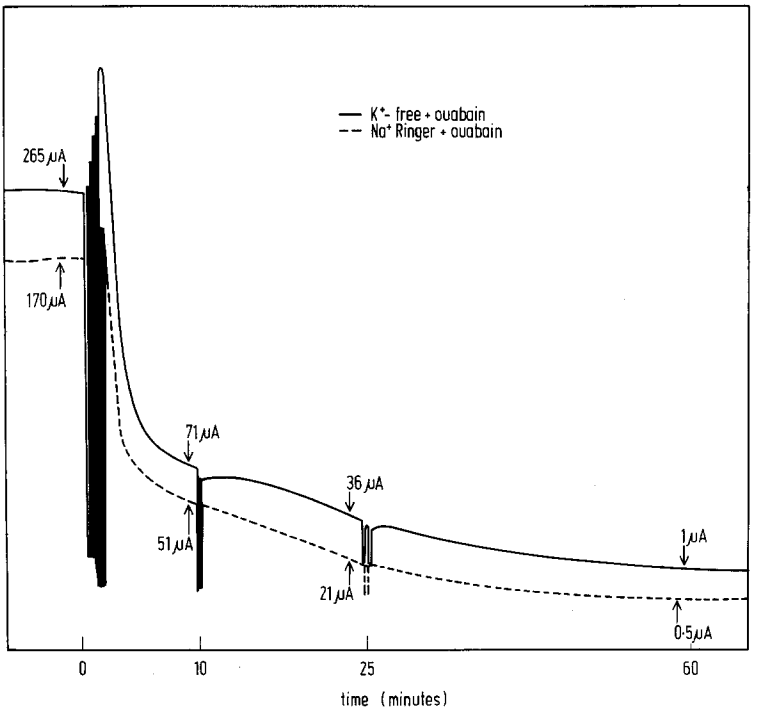

Fig. 5. Effect of ouabain, 10^{-2} M, with either K^+-free Na Ringer's or Na Ringer's serosa. SCC tracing of a pair of hemibladders from Table 7(*c*)

(*vi*) *Effects on Cellular Composition of the Absence of Mucosal Sodium and Serosal Potassium*

Since ouabain inhibited sodium transport and prevented some of the changes seen when serosal potassium was removed, it was possible that inhibiting sodium transport in other ways would also prevent the effects of removing serosal potassium. Choline Ringer's containing 10^{-4} M amiloride was used as a mucosal medium to inhibit SCC, and the effects of then removing serosal potassium were compared with those of removing serosal potassium when the mucosal medium was Na Ringer's (Table 8).

Cells from hemibladders incubated with no mucosal sodium had more potassium and water after removal of serosal potassium than controls with Na Ringer's mucosal medium. The decrease in cellular sodium content with choline Ringer's mucosal medium was that expected in the absence of mucosal sodium—under these conditions, but with a normal serosal potassium concentration, there is a loss of sodium, with a comparable amount of chloride (Macknight *et al.*, 1975*a*). Thus, the similarities of cellular chloride in the present experiments in fact suggest that the cells exposed to mucosal sodium-free solution have lost about 40 mmole/kg dry wt less chloride, as well as less potassium, than have the controls. Mucosal choline Ringer's containing amiloride abolished SCC (SCC was $0 \pm 1\%$, $n=56$) of its previous value after 40 min, and there was no peak of SCC when serosal potassium was subsequently removed (Fig. 6). Experiments with two open-circuited hemibladders likewise showed no transient increases in p.d. on removal of serosal potassium when there was no mucosal sodium.

In another series of experiments, hemibladders were incubated in choline Ringer's containing 10^{-4} M amiloride mucosal media, and then bathed with either K-free Na Ringer's, K-free Na Ringer's containing 10^{-2} ouabain or Na Ringer's serosal media. The results of paired experiments are shown in Table 9. Cellular potassium was lost with K-free media, even in the absence of mucosal sodium; but the losses of potassium with chloride and water were much less than when mucosal sodium was present (Table 1). Under these conditions the compositions of cells exposed to ouabain were no different from those of cells exposed only to K-free serosal medium in contrast to when sodium is available to the cells from the mucosal medium (Table 7). As expected in the absence of mucosal sodium, there was no peak in SCC when serosal potassium was removed simultaneously with the addition of ouabain.

Table 8. Effect on epithelial cellular composition of removal of serosal potassium in the absence of mucosal sodium

Mucosal solution	Cell water (kg/kg dry wt)	Cell ions (mmole/kg dry wt)		
		Na	K	Cl
Na Ringer's	3.03	210	341	154
Choline Ringer's+amiloride	3.32	169	372	151
$\Delta \pm$ SE	0.29 ± 0.12	-40 ± 12	31 ± 14	-3 ± 8
p	<0.05	<0.02	<0.06	>0.60
$n=8$				
(Serosal solution is K-free Na Ringer's)				

Paired hemibladders were mounted in chambers and bathed with Na Ringer's. One of each pair was then exposed to choline Ringer's $+10^{-4}$ M amiloride as mucosal medium, while its pair was bathed with fresh Na Ringer's, on both surfaces. After 40 min of equilibration, ^{14}C-inulin was added to the mucosal medium of both hemibladders. The serosal solution of both hemibladders was then drained and the serosal surface bathed with K-free Na Ringer's+^{3}H-inulin. After 60 min the hemibladders were taken from the chambers, blotted, and the epithelial cells scraped off for analysis.

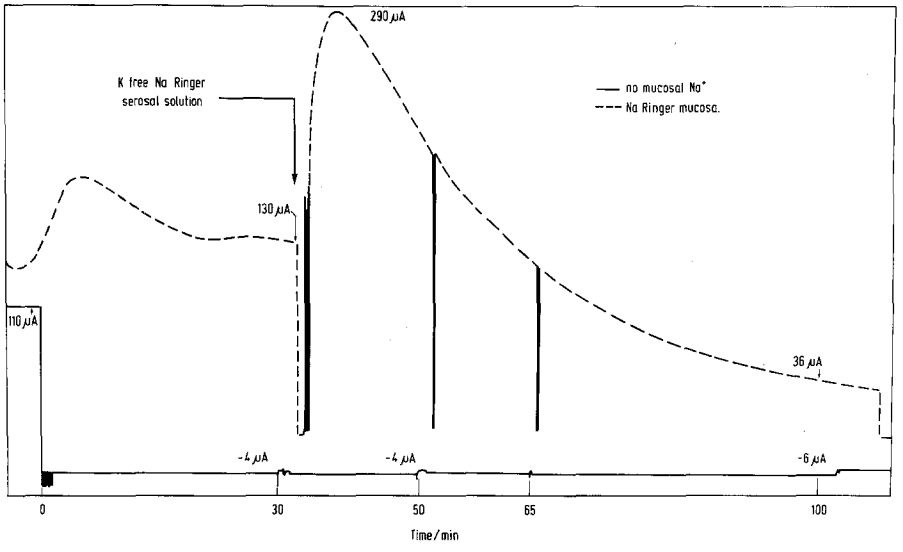

Fig. 6. Effect of removing serosal K when Na is present or absent in mucosal medium. Record of SCC from a typical experiment presented in Table 8

(*vii*) *Effects on Epithelial Cell Composition of Serosal Media with Increased Potassium Concentration*

Epithelial cells from hemibladders which had been incubated in chambers for 60 min in serosal media containing either 7, 14, 48 or 116 mM

Table 9. Effects on epithelial cell composition of potassium-free serosal media and ouabain (10^{-2} M) in the absence of mucosal sodium (Mucosal solution = Choline Ringer's + 10^{-4} M amiloride)

Serosal solution	Cell water (kg/kg dry wt)	Cell ions (mmole/kg dry wt)		
		Na	K	Cl
Na Ringer's	2.90	153	393	199
K-free Na Ringer's	2.83	174	347	166
$\Delta \pm$ SE	-0.07 ± 0.07	21 ± 7	-46 ± 10	-32 ± 6
p	>0.3	<0.05	<0.005	<0.005
$n=7$				
K-free Na Ringer's	2.94	158	363	155
K-free Na Ringer's + ouabain	2.80	166	354	157
$\Delta \pm$ SE	-0.14 ± 0.15	8 ± 7	-8 ± 6	2 ± 7
p	>0.30	>0.30	>0.20	>0.70
$n=8$				

After an initial equilibration in Na Ringer's, short-circuited hemibladders were bathed with choline Ringer's and 10^{-4} M amiloride on their mucosal surfaces. After 40 min ^{14}C-inulin was added to the mucosal medium. Then the serosal chamber was drained and filled with Na Ringer's, K-free Na Ringer's or K-free Na Ringer's + 10^{-2} M ouabain, all with ^{3}H-inulin. After 60 min the hemibladders were taken from the chambers and the epithelial cells collected for analysis.

potassium were analyzed. Paired, control hemibladders were incubated in Na Ringer's (containing 3.5 mM potassium). The cellular compositions are shown in Table 10; the differences in cellular composition between hemibladders incubated in the various serosal potassium concentrations and paired controls in Na Ringer's are shown in Fig. 7. Values for 0 and 1 mM serosal potassium concentrations (from Tables 1 and 4) are included for comparison.

Epithelial cells from hemibladders incubated in a K Ringer's serosal medium gained potassium, chloride and water, and lost sodium relative to control cells from Na Ringer's. About 80 mmole/kg dry wt of potassium was gained in exchange for sodium lost. A further 260 mmole/kg dry wt of potassium was gained with chloride and water in approximately isosmolal proportions.

Incubation in 48 mM K-Na Ringer's serosal medium also resulted in an approximately isosmotic gain of potassium, chloride and water. Cellular sodium content was not significantly changed, although sodium concentration was decreased.

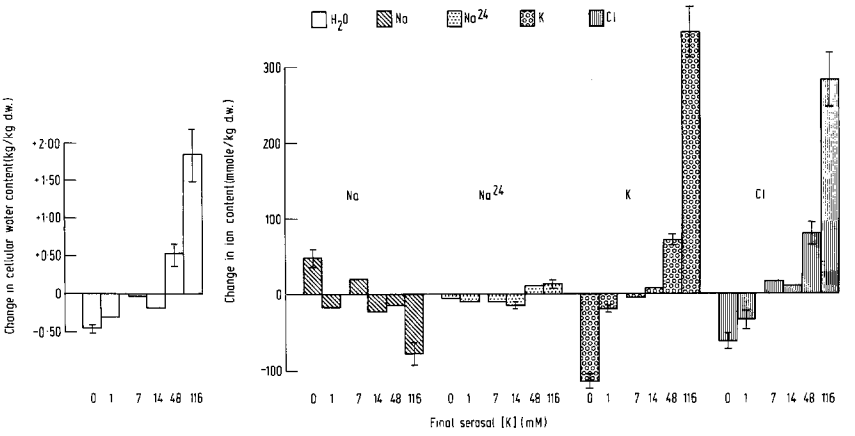

Fig. 7. Change in composition of epithelial cells scraped from bladders exposed to various serosal potassium concentrations relative to controls in 3.5 mM K-Na Ringer's. Summarizes data from Tables 1, 4 and 10. The changes in cellular water and ions, relative to controls from Na Ringer's, are shown for cells scraped from hemibladders incubated 60 min in 7, 14, 48 and 116 mM K (Table 10), 1 mM K (Table 4) and 0 mM K (Table 1) serosal media. The SE is shown where the difference is significant ($p < 0.05$)

With 7 or 14 mM K-Na Ringer's as the serosal medium, no significant changes in cellular sodium, potassium, chloride or water contents were observed.

The changes in cellular composition after incubation in serosal media of low potassium concentration (Tables 1,4) were in the opposite directions to changes after incubation in media of high potassium concentration. There was a close correlation between final cellular potassium and water contents after incubation in 3.5, 7, 14, 48 and 116 mM K serosal media ($r=0.79$, $p<0.001$, $n=62$) and after incubation in K-free Na Ringer's serosal media ($r=0.52$, $p<0.001$, $n=53$).

There were no really significant changes in cellular ^{24}Na content and concentration after incubation in various serosal potassium concentrations. Although cellular ^{24}Na appeared to be increased after incubation in K Ringer's, this increase was barely significant. Cellular ^{24}Na content was apparently decreased after incubation in 14 mM K-Na Ringer's serosal medium. However, the control hemibladders showed an unusually high cellular ^{24}Na content. Thus, this result is unlikely to represent a true decrease in ^{24}Na content. With 48, 7, 1 and 0 mM K-Na Ringer's serosal media, there were no statistically significant changes in ^{24}Na content. Therefore it would seem that changes in serosal

Table 10. Effect on epithelial cell composition of

(*a*) Cellular ion and water content

Serosal solution	Cell water (kg/kg dry wt)	Cell ions (mmole/kg dry wt)			
		Na	^{24}Na	K	Cl
Na Ringer's	2.89	161	40	422	195
K Ringer's	4.73	83	54	766	475
$\Delta \pm$ SE	1.84 ± 0.35	−78 ± 14	14 ± 5	343 ± 32	281 ± 35
p	< 0.001	< 0.001	< 0.05	< 0.001	< 0.001
$n=8$					
Na Ringer's	3.21	173	38	435	199
48 mM K-Na Ringer's	3.74	158	50	506	277
$\Delta \pm$ SE	0.53 ± 0.15	−15 ± 13	12 ± 7	71 ± 6	78 ± 15
p	< 0.01	> 0.2	> 0.05	< 0.001	< 0.001
$n=8$					
Na Ringer's	3.60	216	55	439	251
14 mM K-Na Ringer's	3.43	192	40	445	260
$\Delta \pm$ SE	−0.17 ± 0.15	−23 ± 15	−15 ± 4	7 ± 6	9 ± 18
p	> 0.2	> 0.1	< 0.005	> 0.2	> 0.6
$n=8$					
Na Ringer's	3.43	183	45	427	217
7 mM K-Na Ringer's	3.40	203	36	421	233
$\Delta \pm$ SE	−0.03 ± 0.12	+19 ± 13	−9 ± 4	−6 ± 8	+16 ± 11
p	> 0.8	> 0.1	> 0.05	> 0.4	> 0.1
$n=7$					

Paired hemibladders, mounted in chambers and continuously short-circuited, were equilibrated with Na Ringer's. The mucosal solution included ^{24}Na and ^{14}C-inulin. After 40 min, the serosal chamber was drained and filled with Na Ringer's of a different K

potassium concentration did not alter the size of the mucosal sodium transport pool.

It was possible that the transient changes in SCC on altering serosal potassium concentration were due to potassium diffusion between the cells and serosal medium. Therefore, the numbers of ions represented by the transients in SCC were determined and compared with the changes in cellular potassium for the experiments described in Tables 1, 4 and 10 (Table 11).

The change in cellular potassium was of a similar size to the transient SCC for 0, 48 and 116 mM K media, but not for the other potassium

serosal media of high potassium concentration

(*b*) Cellular ion concentrations

[K] of drained serosal solution (mM)	Cell ion concentrations (mmole/kg cell H_2O)			
	Na	^{24}Na	K	Cl
3.5	55	14	148	68
113	18	12	163	101
	-37 ± 3	-2 ± 1	16 ± 8	32 ± 4
	<0.001	>0.1	<0.05	<0.001
3.5	54	13	136	62
47.9	42	13	137	74
	12 ± 4	1 ± 1	1 ± 5	12 ± 3
	<0.02	>0.5	>0.8	<0.01
3.5	60	16	123	70
13.7	56	12	131	76
	-4 ± 2	-4 ± 1	8 ± 4	6 ± 3
	>0.05	<0.001	>0.05	>0.01
3.6	54	14	125	64
7.2	60	11	124	69
	6 ± 4	-3 ± 1	-1 ± 4	5 ± 3
	>0.1	<0.05	>0.8	>0.1

concentration. The paired hemibladder was bathed with Na Ringer's. These final serosal media contained ^3H-inulin. At 60 min the hemibladders were taken from the chambers and their epithelial cells collected for analysis.

concentrations tested. In addition, the loss of cellular potassium, on removal of serosal potassium, occurred steadily over almost 60 min (Fig. 1) while the transient increase in SCC was completed within 15–20 min. Thus, diffusion of potassium from the epithelial cells could have accounted for only about one-third of the transient increase in SCC under these conditions. This evidence is consistent with evidence presented previously (Robinson & Macknight, 1976*a*) which suggested that movements of an ion or ions other than potassium between the cells and serosal medium were involved in the transients of SCC.

Table 11. Number of ions moving to create transient of SCC

Final serosal [K] (mM)	Ions represented by transient (mmole/kg dry wt)	n	Change in cell K (mmole/kg dry wt)	n
0	-101 ± 16	12	-116 ± 8	12
1	-139 ± 27	7	-17 ± 17	8
7	$+40 \pm 9$	7	-6 ± 8	8
14	$+58 \pm 16$	8	$+7 \pm 6$	8
48	$+125 \pm 35$	8	$+71 \pm 6$	8
116	$+317 \pm 93$	8	$+343 \pm 32$	8

The area of the transient increase or decrease of SCC was estimated using a planimeter, and the number of ions moving per unit area was calculated from the known SCC over a known time interval. The number of ions represented by the transient has been expressed as mmoles of ions in the transient per kg dry weight of cells subsequently scraped from the hemibladder.

The changes in cellular K content of epithelial cells from the same hemibladders, after 60-min incubation in the various K concentrations, are also shown.

– indicates a transient increase in SCC, and a loss of cellular K; + indicates a transient decrease in SCC, and a gain of cellular K.

Discussion

The results of the present experiments showed that when the serosal medium potassium concentration was decreased, toad bladder epithelial cells lost potassium, partly in exchange for sodium from the serosal medium and partly with cellular chloride and water. Cellular sodium of mucosal origin did not change.

Conversely, when the serosal medium potassium concentration was increased substantially, the epithelial cells gained potassium, chloride and water. With K Ringer's serosal solution, the cells showed a loss of cellular sodium of serosal origin, in exchange for serosal potassium.

The Effects of Low Serosal Potassium Concentrations

A nominally potassium-free serosal medium was used in these experiments. However, potassium loss from the tissue during incubation increased serosal potassium concentration, though never above 0.2 mM. It is impossible ever to provide a truly potassium-free solution in contact with the serosal cellular membrane for potassium will be continually diffusing from the cells to the serosal medium. Nevertheless, the changes

in SCC, p.d. and in cellular composition must approach those which would be found under truly potassium-free conditions.

At least half of the transient increase in SCC on removal of serosal medium potassium represented increased M→S Na flux (Robinson & Macknight, 1976*a*). Therefore, the increase in SCC cannot be attributed entirely to an increased net flux of potassium from the epithelial cells to the serosal medium, though such an increase must occur if potassium crosses this membrane by simple passive diffusion. This conclusion is supported by several experimental observations. Firstly, ouabain in K-free Na Ringer's abolished the transient increase in SCC, and resulted in an SCC tracing of slope indistinguishable from that produced by ouabain in the presence of potassium. A transient produced solely by potassium diffusion should have been apparent as a change in slope of the SCC tracing. Secondly, there was no transient increase in SCC or p.d. when hemibladders bathed on their mucosal surfaces by sodium-free choline Ringer's containing 10^{-4} M amiloride were exposed on their serosal surfaces to K-free Na Ringer's. Thirdly, the amount of potassium lost from the hemibladders during the transient increase in SCC could have accounted for only about one third of the increased ion movements that made up the transient. Therefore, increased net potassium diffusion across the serosal membrane from the cells to the serosal medium made only a relatively small direct contribution to the initial increase in SCC and p.d. on removal of serosal potassium.

One possible explanation for this transient is as follows. The interior of an epithelial cell in a short-circuited hemibladder bathed on both surfaces by sodium Ringer's is about 5 mV negative to the media (Frazier, 1962; Reuss & Finn, 1974). Sodium enters the cells passively across the mucosal membrane down both chemical and electrical gradients, and is actively extruded across the serosal membrane (Leaf, 1965). Serosal potassium enters the cell across the serosal membrane as a result of this sodium extrusion. However, as the cell is in a steady state under these conditions the net movement of potassium across this membrane must be zero, and the p.d. across the serosal membrane reflects active M→S transport of sodium.

When serosal medium potassium concentration is suddenly decreased, less potassium can enter the cells, the potassium fluxes across their serosal membranes are no longer equal, and the p.d. across the serosal membrane will reflect not only active sodium extrusion but also net potassium diffusion from the cells to the serosal medium. In the short-circuited state this increased cellular electronegativity would enhance sodium entry

from the mucosal medium to the cells. Since cellular sodium of mucosal origin does not increase (Fig. 1), this increased sodium entry appears to be balanced by a similar increase in sodium extrusion across the serosal membrane, possibly implying a stimulatory effect of increasing cellular sodium concentration on the "sodium pump". However, as discussed in the previous paper, it would seem unlikely that all of the increased current reflects increased M→S sodium movement. Additional current may be carried by potassium lost from the cells. Unfortunately, the sensitivity of the technique does not allow detection of the relatively small change in cellular potassium which might be involved in this process in the early minutes.

How does such a model fit the experimental observations? Ouabain acts on the serosal membrane of toad bladder epithelial cells to block sodium extrusion from the cells. The presence of ouabain in serosal K-free Na Ringer's therefore prevents the increase in M→S sodium transport which would otherwise have occurred, and at the same time profoundly inhibits the existing transepithelial sodium transport. Thus, mucosal sodium entering the cells is not extruded across the serosal membrane but remains within the cells and effectively balances any potassium lost. Therefore the cells do not lose chloride and water. The movement of mucosal sodium into the cells and of cellular potassium to the serosal medium must be balanced by current in the short-circuit pathway. However, the slow decline in SCC to zero which follows the very rapid initial inhibition of SCC by ouabain (Fig. 5) represented the net M→S movement of many more cations than the potassium lost from the cells (Robinson & Macknight, *unpublished observations*), so that this slow fall in SCC cannot simply reflect a slow loss of cellular potassium replaced by mucosal sodium.

Both rubidium (3.5 mM) and choline (116 mM) in the potassium-free serosal medium abolished the initial transient increase in SCC (Robinson & Macknight, 1976*a*). Rubidium presumably diffused into the cells from the serosal medium at a similar rate to that of potassium, for there was a virtual 1:1 exchange of serosal rubidium for cellular potassium with no alteration in cellular water, sodium and chloride (Robinson & Macknight, 1976*b*). Although 3.5 mM choline in the serosal medium did not appreciably modify the initial increase in SCC (Fig. 9, Robinson & Macknight, 1976*a*), 116 mM choline did. This suggests that at relatively high concentrations, enough choline diffused into the cells from the serosal medium to shunt out the increased p.d. resulting from potassium diffusion from the cells across the serosal membrane. Choline gained

by the cells would prevent some chloride loss from the cells with potassium, hence the smaller loss of chloride in K-free choline Ringer's (Fig. 2). Experiments which directly compared cellular potassium after incubation in Na Ringer's or choline Ringer's serosal media, suggest that there was a small loss of potassium, as well as the large loss of sodium, with choline Ringer's. Thus, some cellular potassium may be exchanged for serosal choline (Robinson & Macknight, *unpublished observations*).

However, less potassium was lost after 60 min in K-free choline Ringer's than in K-free Na Ringer's. The reasons for this remain unclear, but it is possible that choline enters the cells from the serosal medium through the same pathways by which potassium is lost, thereby interfering with potassium diffusion from the cells.

The absence of an initial increase in SCC and p.d. when hemibladders equilibrated with sodium-free mucosal media were then exposed to serosal K-free Na Ringer's may simply reflect an insufficient movement of ions across the mucosal cellular membrane to allow detection of electrical events at the serosal membranes of the epithelial cells by electrodes in the mucosal and serosal media. In the absence of mucosal sodium, there can be no increased entry of mucosal sodium to the cells, so that the potential across the serosal membrane, under short-circuited conditions, will not be enhanced by increased extrusion of sodium. Thus, the potential gradient for chloride loss will be considerably less than when increased M→S sodium transport occurred, and the slower rate of chloride loss will limit the rate at which potassium is lost. Thus, after 60 min, cells will contain more potassium, chloride and water than controls with a Na Ringer's mucosal medium (Tables 8,9). Ouabain, under these conditions, should have no effect on cellular composition (Table 9) for there is no mucosal sodium to accumulate in the cells.

Thus, the proposed explanation of the initial increase in SCC and p.d. after removal of serosal potassium accounts for the experimental observations. It can also account for transient increases in SCC whenever serosal potassium concentration is decreased from any initial level.

The Inhibitory Effects of Low Medium Potassium Concentration on Steady SCC and p.d.

The initial increases in SCC and p.d. on decreasing serosal medium potassium concentration were followed by progressive falls in SCC and p.d. to new low steady levels reached by 60 min and maintained for

at least a further 60 min (Robinson & Macknight, 1976*a*). There seemed to be three possible causes of decreased transepithelial sodium transport in serosal media of less than 2 mM potassium. The absence from the serosal medium of potassium itself might have produced the inhibition by a direct effect on the transport mechanism as Koefoed-Johnsen and Ussing (1958) proposed. Alternatively, the inhibition might have resulted either from the loss of cellular potassium or the decrease in cellular volume which accompanied this loss.

Several observations argue against the hypothesis that absence of serosal potassium itself directly inhibits sodium transport when serosal medium potassium is reduced below 2 mM. Firstly, there is evidence against potassium uptake being rigidly coupled to transepithelial sodium transport (Essig & Leaf, 1963; Robinson & Macknight, 1976*b*). Secondly, simple inhibition of the sodium pump, as by ouabain, should inhibit sodium extrusion from the cells and lead to accumulation of mucosal sodium within the cells (Macknight *et al.*, 1975*b*). Even 5×10^{-4} M ouabain, which inhibited SCC after 60 min as much as a potassium-free serosal medium, resulted in a gain of sodium of mucosal origin in exchange for cellular potassium (Table 6). Thirdly, there was less inhibition of p.d. and SCC when K-free choline Ringer's rather than K-free Na Ringer's bathed the serosal surfaces of hemibladders (Figs. 2, 3; Essig & Leaf, 1963). Since choline cannot substitute for potassium in relation to Na-K ATPase activity (Essig, 1965), low potassium concentration in the serosal medium cannot itself directly inhibit SCC and p.d. Fourthly, only a very slow recovery in sodium transport occurred when 3.5 mM potassium was restored to the potassium-free serosal medium (Robinson & Macknight, 1976*a*). Therefore, low serosal medium potassium concentration itself does not directly inhibit sodium transport.

The decrease in cellular potassium, chloride and water on removal of serosal potassium was closely correlated with the extent of inhibition of SCC (Fig. 1). There is some evidence that a decrease in cellular volume might decrease transepithelial sodium transport. Ussing (1965) in frog skin, and Lipton (1972) in toad bladder found that transepithelial sodium transport was inhibited when cells shrank. However, incubation in a hypotonic potassium-free serosal medium failed to prevent the fall in SCC on removal of serosal potassium (Table 3).

Since the loss of chloride is almost certainly due to the loss of potassium from the cells, chloride loss itself is unlikely to be responsible for the inhibition of sodium transport.

However, cellular potassium could play a major role in influencing transepithelial sodium transport, for high potassium concentrations are required for normal functioning of some cellular enzyme systems (Lehninger, 1970). This hypothesis is consistent with the observation that SCC falls much less with K-free choline Ringer's serosal medium, where cellular potassium is less decreased, or when rubidium (3.5 mM) replaces serosal medium potassium. Rubidium may substitute for potassium in its effects on cellular enzyme activity (Lehninger, 1970). This hypothesis is also consistent with the slow restoration of SCC which follows the addition of 3.5 mM potassium to K-free Na Ringer's serosal medium. Preliminary experiments suggest that the restoration of cellular potassium under these conditions is not rapid. Though SCC fell in potassium-free medium, ^{24}Na in the cell did not change. The simplest interpretation of this finding is that ^{24}Na entry at the apical pole of the epithelial cells and its subsequent extrusion from the baso-lateral surfaces of the same cells were proportionately reduced by removal of serosal potassium; but until knowledge of the compartmentation and activity of intracellular sodium is available, no rigorous interpretation of this finding is possible.

The Effects of High Serosal Potassium Concentrations

As described in the preceding paper (Robinson & Macknight, 1976*a*), increasing serosal medium potassium concentration above a level with which hemibladders had previously equilibrated resulted in an initial transient fall in SCC and p.d. followed by a return of SCC to the previous level. With serosal potassium concentrations of 48 and 116 mM, this restoration of SCC was accompanied by a fall in transepithelial p.d. presumably reflecting the increased transepithelial conductance (Robinson & Macknight, 1976*a*).

Epithelial cellular composition was relatively unaffected by 2 of 4 times the normal serosal medium potassium concentration (i.e. 7 and 14 mM potassium), but at higher concentrations cells gained potassium with chloride and water. Only at the highest serosal potassium concentration, 116 mM (with 3.6 mM sodium), did cells lose a significant amount of sodium (Table 10, Fig. 7). Since the chloride concentration is the same in Na Ringer's and K Ringer's, it must be primarily movement of potassium, not chloride, which is responsible for the gains of potassium, chloride and water by the cells.

The transient decrease in SCC and p.d. after exposure to a higher serosal potassium concentration can probably be explained by the con-

verse of the explanation for the initial transient increase in SCC observed when serosal potassium concentration is decreased. Increasing serosal medium potassium will increase influx of potassium to the cell, and decrease the p.d. generated across the serosal membrane by sodium extrusion. The decreased cellular electronegativity, in the short-circuited hemibladder, will reduce the driving force for sodium entry from the mucosal medium and M→S Na flux will fall. The SCC will therefore decrease. Cellular electroneutrality will be preserved by a gain of chloride with the potassium gained; water will follow to preserve cellular osmolarity.

Changes in Cellular Sodium with Variations in Serosal Medium Potassium Concentration

Epithelial cells showed a 1:1 exchange of 50 mmole/kg dry wt of serosal medium sodium for cellular potassium on removal of serosal potassium (Table 1). The exchange was completed within the first 20 min; cellular sodium of serosal origin then remaining stable over the next 100 min (Fig. 1). This exchange is virtually identical to that produced by 10^{-2} M ouabain (Macknight *et al.*, 1975*b*). Such exchange may occur between the serosal extracellular medium and the transporting cells or between medium and cells not engaged in transepithelial sodium transport, or alternatively, between serosal medium and a cellular compartment discrete from the sodium transport pool within the transporting cells. At least some of this exchange probably occurs between basal cells and the surrounding serosal medium.

Several observations favor the hypothesis that serosal sodium does not readily enter the cellular sodium transport pool of the transporting cells. The effects of serosal sodium-free choline Ringer's on p.d. and SCC, and effects of ouabain and of aldosterone on toad bladder oxygen consumption have been discussed in detail elsewhere (Macknight *et al.*, 1975*a*). The preceding paper (Robinson & Macknight, 1976*a*) demonstrated the ineffectiveness of serosal 116 mM sodium in shunting the transient increase in p.d. and SCC which accompanies reduction of serosal medium potassium, when compared with substitution of 3.5 mM rubidium or caesium for serosal potassium, or of 116 mM choline or lithium for serosal sodium. This suggests that the serosal membrane of the cell is much less permeable to sodium than to these other cations. Furthermore, the recent suggestion that the specific resistance of the serosal membrane, is an order of magnitude greater than that of the mucosal membrane

(Reuss & Finn, 1974) is not consistent with a model which considers sodium labelling of the cells to be a result of sodium entering 4 times faster across the serosal membrane than across the apical membrane (Macknight *et al.*, 1975*a*). It is of interest that the serosal membranes of the epithelial cells lining the alimentary canel (Schultz, Frizzel & Nellans, 1974) and of the epithelial cells in frog skin (Ussing, Erlij & Lassen, 1974) may also be relatively impermeable to sodium.

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