J. Membrane Biol. 26, 217–238 (1976)

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

I. Effects of Different Medium Potassium Concentrations on Electrical Parameters

Bridget A. Robinson and Anthony D.C. Macknight

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

Received 11 April 1975; revised 31 July 1975

Summary. When serosal medium potassium was decreased from the usual concentration of 3.5 mM, the short-circuit current (SCC) of hemibladders in chambers immediately and transiently increased. The maximum SCC attained was greater the greater the decrease in serosal potassium, and was twice the initial SCC when the final serosal medium was potassium-free. The SCC then fell to its previous level for final serosal potassium concentrations greater than 2 mM and to less than its previous level for those less than 2 mM, being lowest (15% of previous level) in potassium-free sodium Ringer's. When serosal medium potassium was increased from 3.5 mM by substituting potassium for sodium, SCC transiently decreased and then recovered to its previous level. Steady SCC was the same in serosal media of 2–116 mM potassium; conductance increased and p.d. decreased after incubation in 50–116 mM potassium serosal media. Short-circuit current and p.d. transiently increased (decreased) whenever serosal medium potassium was decreased (increased); conductance increased with any change in serosal potassium. Changing mucosal medium potassium concentration between 0 and 50 mM did not affect SCC. The initial transient increase and subsequent decrease in SCC on removing serosal potassium were partially prevented by 3.5 mM rubidium or caesium, or by 116 mM choline in the serosal medium. The transient changes in SCC were due partly to changes in transepithelial sodium transport.

The effects of medium potassium on transepithelial sodium transport remain poorly understood. Many epithelia seem to require the presence of potassium, in a concentration similar to that of the extracellular fluid, in the media bathing the surface towards which sodium is being transported (frog skin, Koefoed-Johnsen & Ussing, 1958; toad bladder, Bentley, 1959; Hays & Leaf, 1961; fish gallbladder, Diamond, 1962). However, the mechanism by which medium potassium supports transepithelial sodium transport remains controversial. One possibility, first advanced by Koefoed-Johnsen and Ussing (1958) and Ussing (1960) is that there is an obligatory requirement for potassium to exchange for sodium across

the baso-lateral membranes of the cell as an integral part of the functioning of the sodium pump. In the absence of serosal potassium, the transepithelial transport of sodium, and also any serosal exchange of sodium for potassium, would be inhibited. A second possibility, proposed by Essig and Leaf (1963) as a result of experiments in which the composition of whole toad hemibladders was determined, is that removal of potassium from the serosal medium inhibits entry of sodium from the mucosal medium to the cells, thereby inhibiting transepithelial sodium transport.

The recent development of techniques whereby epithelial cells alone can be obtained and analyzed after toad hemibladers have been incubated in vitro (Macknight, DiBona, Leaf & Civan, 1971) should allow a more precise analysis of this problem than has previously been possible. We therefore decided to reinvestigate the role of K in transepithelial sodium transport in toad bladder.

However, it became apparent as this investigation began, that dramatic though transient changes in potential difference and short-circuit current accompanied changes in serosal medium potassium concentration. Since such changes were only poorly documented in the literature (Essig, 1965; Finn, Handler & Orloff, 1967) experiments were performed to examine these transients in greater detail and were extended to investigate the effects which variations in medium potassium concentration had on the final steady p.d. and SCC in toad bladder.

This paper presents the results of these experiments. Subsequent papers (Robinson & Macknight, 1976*a,b*) present results showing the effects of variations of serosal medium potassium concentration on cellular composition and on the exchangeability of cellular potassium with mucosal and serosal potassium.

Materials and Methods

Female toads of the species *Bufo marinus* were obtained from the Dominican Republic (National Reagents, Bridgeport, Conn.) and were kept on woodshavings with free access to water. Toads were doubly pithed and the beating heart perfused with toad Ringer's solution. The deblooded hemibladders were dissected from the abdominal cavity and spread between the two halves of Ussing-type chambers (exposed surface area 8.04 cm^2). Initially both halves of the chambers were filled with sodium Ringer's solution (Na Ringer's, Table 1). Air, bubbled through the media, provided oxygenation and stirring. All experiments were performed at room temperature (18–22 °C).

The compositions of the media used are shown in Table 1. Media with potassium concentrations between 3.5 and 116 mM (K Ringer's) were prepared by substituting potassium for sodium in Na Ringer's; media with potassium concentrations between 0 and 3.5 mM were prepared by substituting sodium for potassium. Sometimes 3.5 mM of the

Table 1. Composition of Ringer's solutions used

	Na^+	K^+	Ca^{++}	Cl^-	HPO_4^{2-}	Glucose	Others
	(Concentration, mmole/liter solution)						
Na Ringer's	115.6	3.5	1.0	117.5	1.8	10	
K-free Na Ringer's	119	–	1.0	117.5	1.8	10	
7 mM K-Na Ringer's	112	7.0	1.0	117.5	1.8	10	
14 mM K-Na Ringer's	105	14.0	1.0	117.5	1.8	10	
35 mM K-Na Ringer's	84	35	1.0	117.5	1.8	10	
48 mM K-Na Ringer's	71	48	1.0	117.5	1.8	10	
60 mM K-Na Ringer's	59	60	1.0	117.5	1.8	10	
K Ringer's	3.6	115.6	1.0	117.5	1.8	10	
Choline Ringer's	–	3.6	1.0	117.5	1.8	10	112, choline
K-free chol. Ringer's	3.6	–	1.0	117.5	1.8	10	116, choline
Lithium Ringer's	3.6	3.5	1.0	117.5	1.8	10	112, lithium
K-free Li Ringer's	3.6	–	1.0	117.5	1.8	10	116, lithium
3.5 mM Rb-Na Ringer's	115.6	–	1.0	117.5	1.8	10	3.5, rubidium
3.5 mM Cs-Na Ringer's	115.6	–	1.0	117.5	1.8	10	3.5, caesium
3.5 mM Li-Na Ringer's	115.6	–	1.0	117.5	1.8	10	3.5, lithium
3.5 mM chol. Na Ringer's	115.6	–	1.0	117.5	1.8	10	3.5, choline

All media also contained 100 mg/liter benzylpenicillin (Dista Products Ltd.) and 50 mg/liter streptomycin sulfate (Glaxo Laboratories (NZ) Ltd.). Medium pH was 7.8; osmolarity was 220–230 mosm/liter.

various alkali cations or of choline replaced potassium in Na Ringer's. In choline Ringer's and lithium Ringer's serosal sodium was replaced by choline or lithium, respectively.

Once hemibladders were mounted in chambers and bathed with Na Ringer's, the transepithelial p.d. was recorded, and then, in most experiments, the hemibladders were continuously short-circuited. When the SCC was steady, media were replaced by Ringer's solutions modified by alterations in their sodium and potassium concentrations. The chambers were washed through with the new media before being filled. Hemibladders were incubated in the new media until SCC was steady. Then the hemibladders were incubated either with another modified medium, or with Na Ringer's again. In many experiments the paired hemibladder was used as a control and was incubated in Na Ringer's throughout and washed with fresh Na Ringer's when the media bathing the experimental hemibladder were changed. In other experiments a hemibladder was exposed successively to many media of different potassium concentrations. The results in such experiments were accepted if the SCC when hemibladders were reincubated in Na Ringer's was similar to that found with Na Ringer's at the beginning of the experiment. The hemibladder thus served as its own control.

Medium potassium concentrations were determined flame photometrically in all experiments. Electrical contact between the media in the chambers and electrodes was made using 4 M sodium acetate agar leads. These leads were used rather than the more conventional 3 M potassium chloride leads because preliminary experiments had shown a considerable loss of potassium from the potassium chloride leads to the media (Fig. 1). Experiments in which hemibladders were bathed by Na Ringer's showed that this substitution of 3 M potassium chloride leads by 4 M sodium acetate leads did not affect the measured SCC or p.d. When a potassium-free medium was used, the chamber was first washed out 5

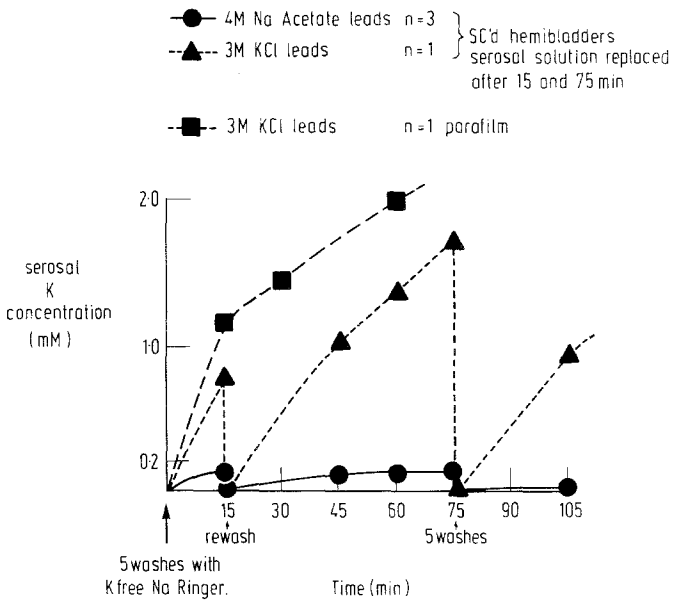

Fig. 1. The effects on medium potassium concentration of incubating hemibladders in chambers with 3 M potassium chloride leads or 4 M sodium acetate leads. At time 0, K-free Na Ringer's was added to the serosal chambers (after 5 washes). Serosal medium potassium concentration was determined after various time intervals. The serosal medium was washed out after 15 and 75 min. In addition, medium potassium concentration was measured when 3 M potassium chloride leads were used and K-free Na Ringer's bathed both surfaces of parafilm mounted in the chamber

times with the potassium-free solution and then filled. The chamber was rewashed 15 and 30 min later in order to keep medium potassium always less than 0.2 mM.

Transepithelial SCC was continuously recorded in most experiments. Sometimes, transepithelial p.d. and conductance were recorded after equilibration in the various media, and also immediately after the bathing solutions were changed. Hemibladders were briefly open-circuited to record p.d.; conductance was then calculated as the ratio of the SCC to the p.d. Alternatively, conductance was measured by applying 10-mV potentials across the hemibladders at regular time intervals and recording the change in SCC; conductance was the ratio of the change in SCC to the potential applied. In some experiments hemibladders were continuously open-circuited—then only p.d. was recorded.

With some medium potassium concentrations the unidirectional flux of isotopic ^{24}Na from the mucosal to the serosal medium (M→S) was studied. Modified, continuous flow chambers (of volume 2 ml) were used to contain the serosal medium. The effluent from the flow chamber was collected as one-minute fractions, at a flow rate of 5–10 ml/min. Initially the hemibladders were bathed by Na Ringer's and continuously short-circuited. Isotopic ^{24}Na (obtained from Australian Atomic Energy Commission, Lucas Heights, New South Wales) was then added to the mucosal Na Ringer's. The effluent from the serosal chamber was discarded during the first 20 min to allow ^{24}Na to reach a steady distribution through the tissue and to allow SCC to stabilize. Then samples of effluent were collected for 10–20 min. The serosal medium was then changed, and samples of effluent collected for 30–60 min. Samples of the mucosal medium, and all the serosal medium fractions

were counted in a gamma scintillation spectrometer (Packard Instrument Company, Inc.) so that the flux of ^{24}Na across the bladder could be determined. The volume of each one-minute fraction was determined gravimetrically.

The final steady values of SCC, p.d. and conductance recorded after a hemibladder had been incubated in a modified medium are expressed as percentage of their initial steady values before exposure to that medium. Often the initial medium was Na Ringer's, so that SCC, p.d. and conductance could be expressed as percentages of the steady values in Na Ringer's. Results are presented as mean $\pm$SEM. Groups of observations were compared using Student's t-test for unpaired data.

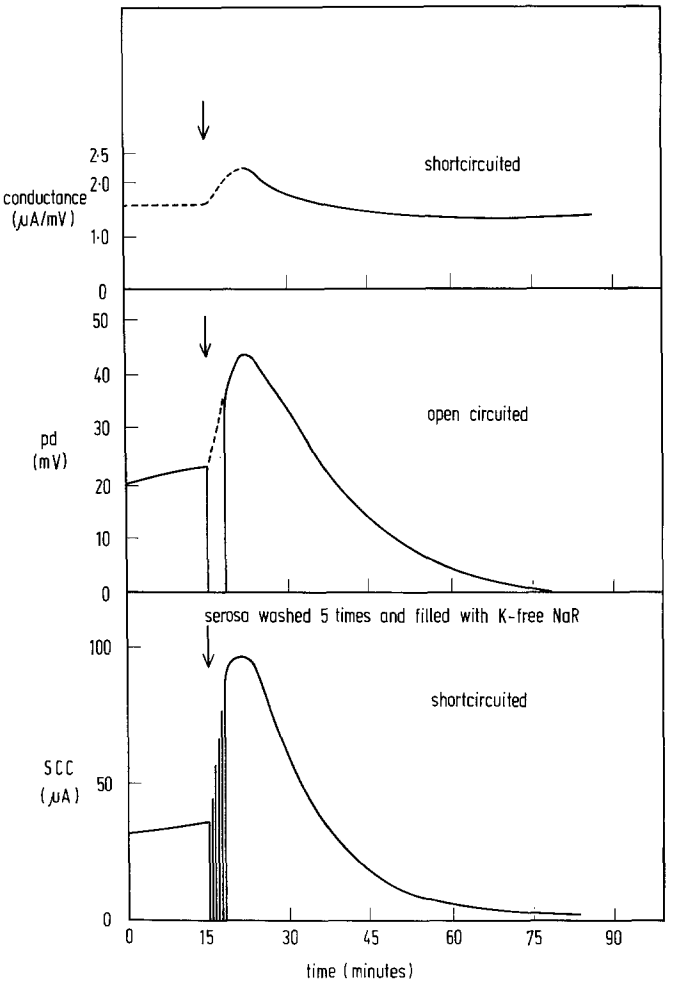

Fig. 2. Effect of potassium-free serosal medium on the short-circuit current and conductance of a short-circuited toad bladder, and on the potential difference of its open-circuited pair. A pair of hemibladders was equilibrated in Na Ringer's and then exposed to K-free Na Ringer's serosal medium. One hemibladder was short-circuited and the other open-circuited throughout the experiment. Conductance was measured by recording the change in SCC when a known potential was applied across the short-circuited hemibladder

Results

(*i*) *Effects on SCC of Potassium-free Serosal Media*

Experiments were performed to examine in more detail the effects on SCC of removing serosal potassium. Fig. 2 shows a typical result when hemibladders equilibrated with Na Ringer's were bathed on their serosal surfaces with nominally K-free Na Ringer's. The SCC did not fall immediately, but instead increased to reach a maximum value within 2–5 min. It then fell steadily, passing through its initial value within 15–20 min. A similar peak was observed in the p.d. of open-circuited hemibladders. Conductance increased somewhat during the peak of SCC. In 58 experiments, the peak of SCC averaged $200 \pm 6\%$. The peak of p.d. of open-circuited hemibladders was $162 \pm 9\%$ ($n=6$).

After the initial transient increase in SCC on removal of serosal potassium, SCC fell to reach a new low, but relatively steady, SCC after about 60 min which averaged 15% of the initial SCC in Na Ringer's (Table 2). There was no further drop in SCC over the next hour. Only in a few hemibladders did SCC actually become zero.

Experiments were also performed to examine the recovery in SCC when the serosal K-free Na Ringer's that had bathed hemibladders for

Table 2. Effects of K-free Na Ringer's serosal medium on SCC and p.d. of hemibladders

A. Short-circuited hemibladders				
M medium	S medium	Incubation time (min)	% final SCC / SCC in NaR	*n*
NaR	Na Ringer's	60	107 ± 2	85
	K-free Na Ringer's	60	15 ± 1	97
	K-free Na Ringer's	120	16 ± 2	32

B. Open-circuited hemibladders				
M medium	S medium	Incubation time (min)	% final p.d. / p.d. in NaR	*n*
Na Ringer's	Na Ringer's	60	112 ± 3	7
	K-free Na Ringer's	60	15 ± 6	6

The SCC and p.d. are expressed as percentages of the steady SCC or p.d. with Na Ringer's as mucosal and serosal media, before the hemibladders were bathed in the media indicated. All values are means ± SEM.

60 min was replaced with Na Ringer's. In 15 hemibladders, SCC recovered to $101 \pm 11\%$ of the initial SCC in Na Ringer's, but this recovery took well over 2 hr. Indeed, immediately serosal potassium was replaced, SCC actually decreased even further before the recovery began. Such behavior is consistent with the results presented below which show that SCC normally decreases initially whenever hemibladders are exposed to a potassium concentration higher than that with which they have equilibrated.

(*ii*) *Effects on SCC of Low Serosal Potassium Concentrations*

Whenever serosal potassium concentration was decreased to virtually 0 mM, SCC immediately increased to a peak value before decreasing. It was therefore possible that smaller decreases in serosal potassium concentration would also result in immediate peaks of SCC. In addition, the SCC after 60 min might be less inhibited if the serosal potassium concentration were not decreased as much.

To investigate this possibility, hemibladders, mounted in chambers and continuously short-circuited, were equilibrated with Na Ringer's. Then the serosal chambers were drained and filled by media of potassium concentrations between 0 and 3.5 mM. The mucosal medium was Na Ringer's throughout the experiments. The hemibladders were bathed with each low potassium medium until SCC stabilized (less than 60 min). The results are shown in Fig. 3 (with results to be presented later from experiments in which high serosal potassium concentrations were employed). The "final SCC" is the steady SCC in the final serosal potassium concentration indicated along the abcissa; "transient SCC" is the maximal (or minimal) SCC attained a few minutes after decreasing (or increasing) the serosal potassium concentration. With serosal potassium above about 2.0 mM the final steady SCC seemed independent of serosal potassium concentration. With serosal potassium less than 2.0 mM, the lower the serosal potassium concentration the lower the final SCC. Between 3.5 and 1 mM, the peak SCC on reducing serosal potassium concentration was greater the greater the decrease in serosal potassium concentration.

When the serosal surface of hemibladders which had equilibrated with low potassium serosal media were then bathed with K-free Na Ringer's, SCC again increased initially before falling further. It was found that the greater the decrement in serosal potassium concentration, the greater the initial peak in SCC.

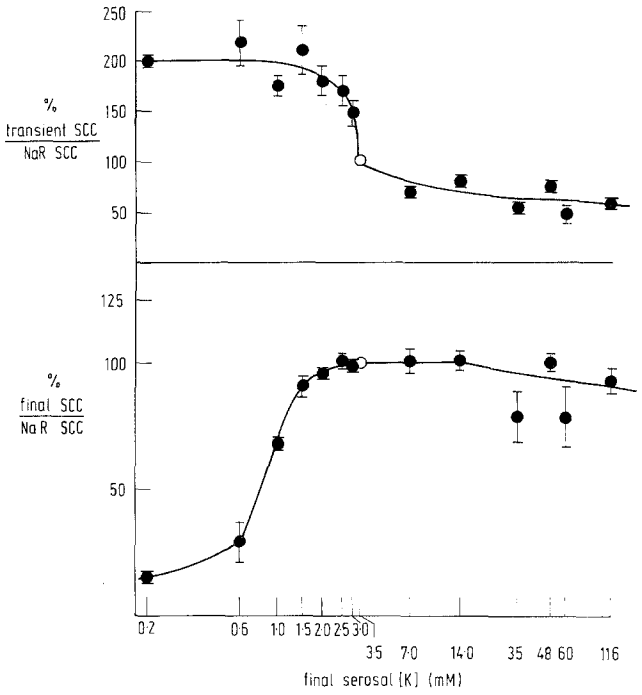

Fig. 3. Effect of changing serosal K concentration from 3.5 mM. Hemibladders equilibrated with Na Ringer's were then bathed with serosal media of potassium concentrations ranging from 0 to 116 mM. The initial transient SCC and final steady SCC, on changing serosal potassium concentration to the values along the abscissa, are expressed as percentages of the previous SCC in Na Ringer's. $n > 5$; means $\pm$ SEM

(*iii*) *Effects on SCC of High Serosal Potassium Concentrations*

An initial increase in SCC was characteristic of decreasing serosal potassium concentration from its usual value of 3.5 mM. By analogy, if serosal potassium concentration were increased, it seemed possible that an initial decrease in SCC would occur. Experiments were therefore performed in which hemibladders were exposed to serosal media with potassium concentrations greater than 3.5 mM.

Fig. 4 shows the SCC from a typical experiment in which a hemibladder equilibrated with Na Ringer's was then bathed with a 14 mM K-Na Ringer's serosal solution. Fig. 5 shows the SCC with incubation in a K Ringer's serosal solution. Immediately, the hemibladders were exposed to the higher potassium serosal media, SCC dropped rapidly, reached a minimum value, and then slowly increased. A similar transient decrease in SCC occurred whenever serosal potassium concentration was

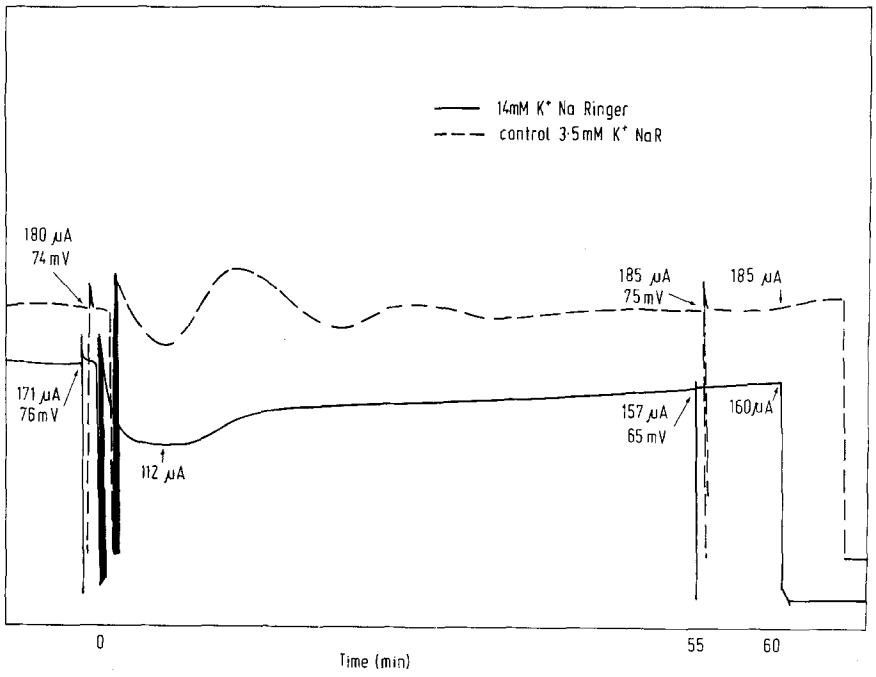

Fig. 4. Effect on SCC of 14 mM K-Na Ringer's on serosal side of a toad bladder previously exposed to 3.5 mM K-Na Ringer's. Both hemibladders were initially equilibrated in Na Ringer's. At zero time, the serosal medium bathing one hemibladder was replaced by 14 mM K-Na Ringer's, while fresh Na Ringer's bathed the other. The hemibladders were incubated 60 min in these media. Transepithelial p.d. and conductance were recorded at 0 and 55 min by briefly open-circuiting the hemibladders

increased above that with which the hemibladder had been equilibrated. In contrast, control hemibladders bathed with Na Ringer's, although sometimes showing variations in SCC, never displayed any consistent changes when the serosal medium was drained and replaced with fresh Na Ringer's.

Fig. 3, in addition to showing the effects of low serosal medium potassium, summarizes the results obtained when hemibladders equilibrated with Na Ringer's were exposed to serosal media containing more than 3.5 mM potassium and incubated until SCC was again steady. In all experiments, despite greater concentrations, SCC recovered towards the initial value it had had in Na Ringer's.

In additional experiments it was found that at higher serosal potassium concentrations, doubling or halving serosal potassium caused smaller transients, as percentages of the initial steady SCC's, than at serosal potassium concentrations between 3.5 and 35 mM. However, these

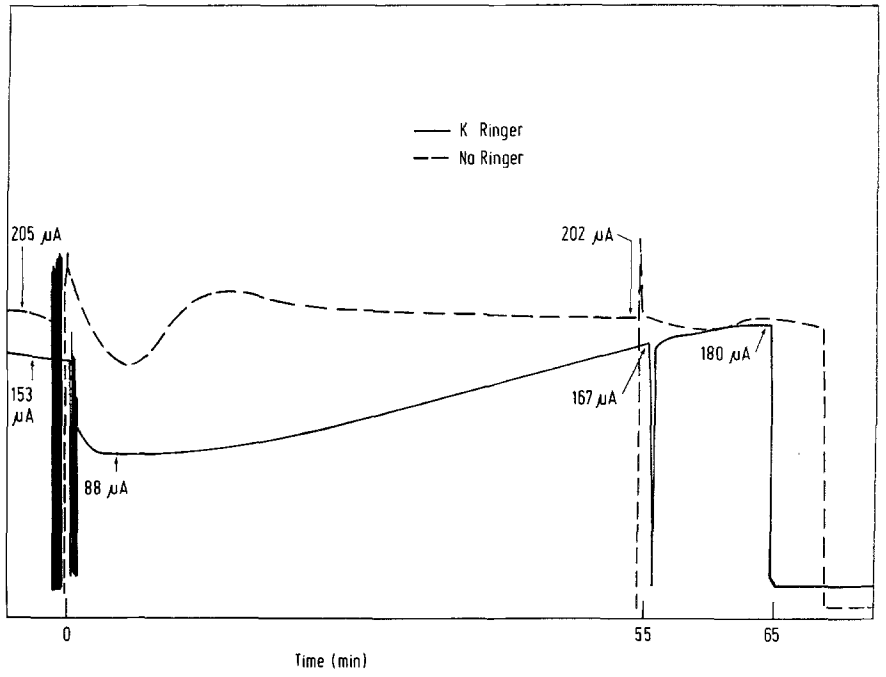

Fig. 5. Effect on SCC of K Ringer's on serosal side of a toad bladder previously exposed to 3.5 mM K-Na Ringer's. Record of SCC from a typical experiment in which, at zero time, K Ringer's replaced Na Ringer's as the serosal medium. The paired control hemibladder had a Na Ringer's serosal medium throughout

transients occurred more slowly, and SCC recovered more slowly, than at lower serosal potassium concentrations. A slower, more prolonged transient would represent the net movement of more ions than a briefer transient with the same percentage change in SCC.

Further studies revealed that whatever the serosal medium potassium with which the hemibladder had equilibrated, an increase in serosal potassium concentration caused a transient decrease in SCC, while a decrease in serosal potassium concentration caused a transient increase in SCC. For serosal potassium concentrations greater than 3.5 mM, steady SCC seemed relatively unaffected by changes in serosal potassium concentration.

(*iv*) *Effects on p.d. and Conductance of Changes in Serosal Medium Potassium Concentration*

Transepithelial SCC, p.d. and conductance were all measured for each of a number of hemibladders before and after equilibration at

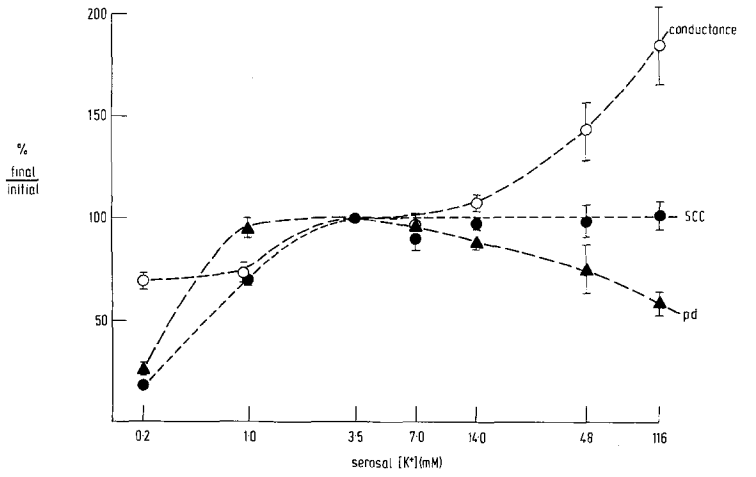

Fig. 6. Transepithelial SCC, p.d. and conductance at various serosal K concentrations. Hemibladders equilibrated with Na Ringer's were then incubated 60 min (or 120 min for 0.2 mM K-Na Ringer's results) in media of various serosal potassium concentrations. The graph shows the final steady SCC's, p.d.'s and conductances, as percentages of their initial values in Na Ringer's (mean $\pm$ SE, $n=7$)

various serosal potassium concentrations (Fig. 6). At serosal potassium concentrations between 3.5 and 14 mM there was little change in steady SCC, conductance and p.d. In contrast, although SCC was maintained by hemibladders incubated in 48 and 116 mM potassium, transepithelial p.d. decreased and conductance increased. Indeed, conductance was almost doubled by incubation in a K Ringer's serosal medium. When serosal potassium concentration was decreased to 1 or 0 mM, SCC, p.d. and conductance were all reduced.

(*v*) *Effects on SCC of Changes in Mucosal Potassium Concentration*

All experiments described so far were performed by changing the composition of the serosal media only, leaving Na Ringer's bathing the mucosal surfaces of the hemibladder. Under these conditions, the gradient for passive diffusion of potassium across the hemibladder will be altered. It is therefore important to exclude alterations in transepithelial potassium flux as an explanation for the observed changes in p.d. and SCC.

If the transient changes in SCC were due to changes in net transepithelial potassium diffusion, changing both mucosal and serosal potassium concentrations to the same extent at the same time should abolish these

transients. In a series of experiments, however, it was found that the effects of both increasing or decreasing medium potassium concentrations on both mucosal and serosal surfaces together were indistinguishable from the effects of changing serosal medium potassium concentration alone.

In addition, if the transient changes in p.d. and SCC associated with alterations in serosal potassium concentration resulted from transepithelial potassium diffusion, changing only mucosal medium potassium concentration should have produced transient changes in p.d. and SCC of the same magnitude but opposite orientation to those observed when only serosal potassium concentration was altered. However, when the mucosal potassium concentration was varied between 0 and 48 mM no significant changes in SCC were observed ($n=8$). (Higher mucosal medium potassium concentrations could not be tested with a normal medium osmolarity, because the necessary decrease in mucosal medium sodium concentration would itself decrease transepithelial sodium transport.)

These experiments demonstrated that, with potassium concentrations less than 48 mM, the transients of SCC associated with alterations in serosal potassium concentration were not simply the result of changes in net passive transepithelial potassium diffusion. It is, however, possible that the increased conductance in hemibladders equilibrated with higher potassium concentrations might be associated with some contribution of transepithelial potassium flux to the transient depression in SCC observed under these conditions.

In addition, observations were made which excluded the possibility that the transients of SCC observed whenever serosal potassium concentration was changed, were due simply to alterations in hemibladder physiology resulting from the draining and refilling of the serosal chamber.

(*vi*) *Effects on M→S Sodium Flux of Changes in Serosal Potassium Concentration*

Since in the experiment described above, media of different composition bathed the mucosal and serosal surfaces of the hemibladders, the measured SCC did not necessarily reflect net M→S sodium flux. Therefore the unidirectional M→S sodium flux was determined, simultaneously with SCC, when hemibladders equilibrated with Na Ringer's were then

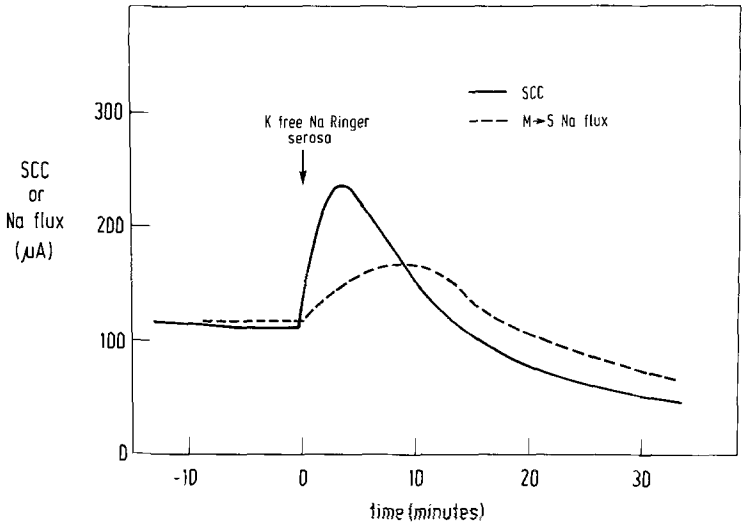

Fig. 7. Effect on SCC and on M→S Na flux of removal of serosal K. The simultaneous SCC and M→S Na flux of a hemibladder initially equilibrated with Na Ringer's, and then at time zero, bathed with a K-free Na Ringer's serosal medium. The serosal medium flowed continuously through the serosal chamber

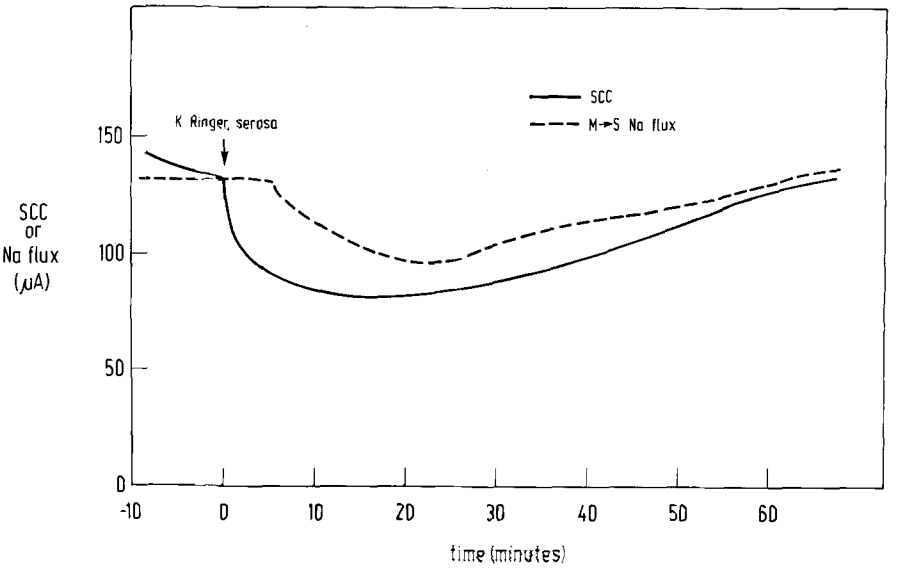

Fig. 8. The simultaneous SCC and M→S Na flux of a hemibladder initially equilibrated with Na Ringer's, and then at time zero, bathed with a K Ringer's serosal medium. The serosal medium flowed continuously through the serosal chamber

exposed to K Ringer's or K-free Na Ringer's serosal media. The results are shown in Figs. 7 and 8.

When hemibladders were incubated in Na Ringer's, M→S Na flux closely approximated SCC. On removal of serosal potassium (Fig. 7), the M→S Na flux showed an initial transient increase, as did SCC. The peak in M→S Na flux was delayed relative to the peak in SCC, presumably as a result of the inevitable delay in ^{24}Na extruded from the epithelial cells reaching the fraction collector. Because of the lag in the appearance of ^{24}Na it is difficult to quantitate the increase in SCC with the increase in ^{24}Na flux. If one calculates the area under the curves for SCC and for ^{24}Na flux until the values of each return to their control levels, then the ^{24}Na flux accounts for about 50 per cent of the transient increase in SCC. However, the higher value for the ^{24}Na flux, when compared with SCC, for the remainder of the experiment indicates that such a calculation underestimates the contribution of M→S Na transport to the transient increase in SCC. This conclusion is supported by the reduction in conductance which invariably occurs in K-free media.

When a hemibladder was bathed by a K Ringer's serosal medium, the SCC promptly decreased to 60% of its initial value (Fig. 8). After a brief delay, M→S Na flux also decreased, and then slowly recovered. The decrement in M→S Na flux accounted for only 43% of the whole decrement in SCC in this experiment. Again, this estimate must be qualified because of the difficulties in accurately determining the total area of the ^{24}Na curve which is associated with the decreased SCC.

(*vii*) *Effects on SCC of Substitution of Other Cations for Serosal Potassium*

The specificity of serosal potassium in supporting SCC in toad hemibladders was examined by substituting rubidium, caesium, lithium or choline, all at a concentration of 3.5 mM, for potassium in the serosal medium. The results of these experiments are summarized in Fig. 9, which also shows for comparison results, already presented, of substitution in the serosal medium of 3.5 mM sodium for potassium, i.e. K-free Na Ringer's.

The lowest graph in Fig. 9 shows the effects on steady SCC of substituting another cation for serosal potassium. The various cations differed in the degree to which they enabled SCC to be maintained. The middle graph illustrates the effect of the substitution on the transient increase in SCC observed immediately when Na Ringer's was replaced. Compari-

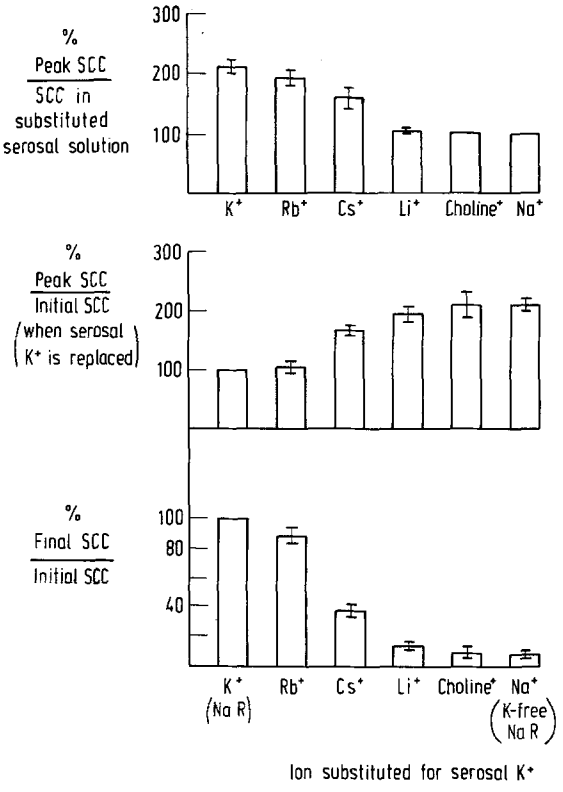

Fig. 9. Effect of replacing serosal K by Rb, Cs, Li, Na and choline. All K in serosal Na Ringer's was replaced by 3.5 mM of the ion indicated along the abscissa. The final steady SCC attained is shown in the lowermost graph. The initial transient peak of SCC on changing the serosal medium is shown in the middle graph. The uppermost graph presents the peak of SCC on replacing the substituted serosal medium by K-free Na Ringer's (in which Na substitutes for K). $n > 5$, mean $\pm$ SEM

son of the two lower graphs of Fig. 9 shows that the greater the peak of SCC immediately as serosal potassium was replaced, the lower the final steady SCC achieved.

The uppermost graph (Fig. 9) shows the transient increase in SCC when K-free Na Ringer's replaced the substituted Na Ringer's with which the hemibladders had been equilibrated. The greater the steady SCC when another alkali cation had substituted for potassium, the greater the peak SCC on subsequently changing to a K-free Na Ringer's serosal medium.

In these experiments, the two surfaces of a hemibladder were bathed by media of different composition; thus SCC did not necessarily represent transepithelial sodium flux. However, in a series of eight experiments

M→S Na flux was found to be equivalent to SCC both before and after substitution of serosal potassium by 3.5 mM rubidium.

(*viii*) *Effects on SCC of Substitution of Serosal Sodium by Other Cations*

The changes in SCC observed with changes in serosal potassium concentration could have been due to changes in serosal sodium concentration. In particular, the SCC response to a K Ringer's serosal medium could have been the result of the removal of all serosal sodium. Therefore all serosal sodium was replaced by lithium or choline and the effect on SCC compared with that of a K Ringer's serosal medium. The results are presented on the left in Fig. 10. (Results for K Ringer's were also presented in Fig. 3; more detailed results for choline Ringer's are presented in the next paper, Robinson & Macknight, 1976*a*.) The change in SCC on adding a K Ringer's serosal medium was clearly

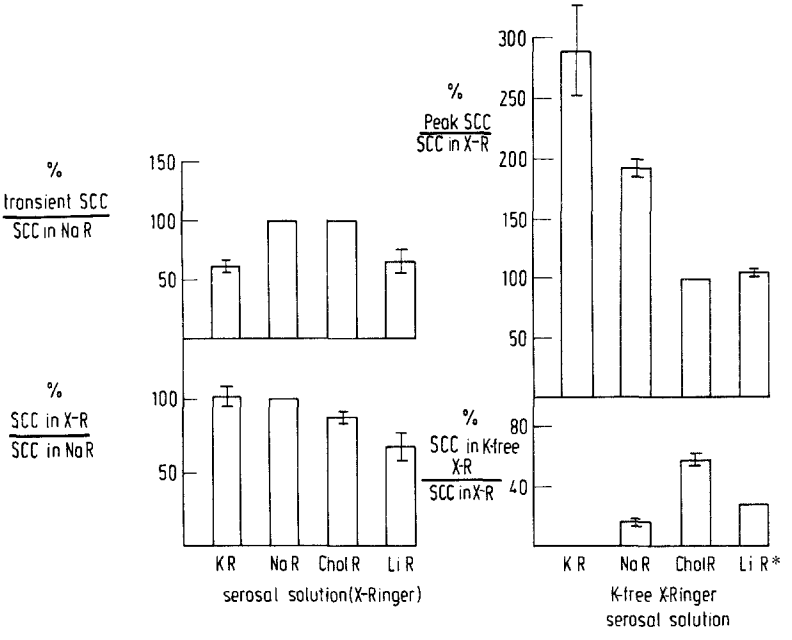

Fig. 10. Effects on SCC of replacing serosal Na by K, Li or choline; and then removing K. The graphs on the left illustrate the transient and steady SCC's after serosal Na Ringer's was replaced by the media shown. The graphs on the right illustrate the peak and final steady SCC's when the serosal media were made potassium-free. Steady SCC's were recorded usually 60 min after the serosal media were changed. $n>5$; * $n=2$

different from changes in SCC on replacing all serosal sodium by lithium or choline. Thus, the SCC responses to changes in serosal potassium concentration seemed to be specific for potassium.

All potassium was then removed from these substituted serosal media; the resultant initial increases in SCC and final steady SCC's are shown on the right in Fig. 10. As has previously been observed, greater initial peaks of SCC were associated with greater final depression of SCC. From Fig. 10, choline Ringer's and lithium Ringer's both seemed to prevent the inhibition of SCC on removal of serosal potassium, choline being more effective than lithium.

Discussion

The results presented above may be summarized as follows. Whenever the potassium concentration in the serosal medium was decreased below that with which the hemibladders had been equilibrated, SCC (and p.d.) transiently increased and then fell. If the potassium concentration in the new medium was greater than about 2 mM, SCC and p.d. returned to their previous values. However, if the potassium concentration in the new medium was less than 2 mM, SCC and p.d. fell below the previous values to some new steady value which, even with a nominally potassium-free serosal medium, was usually greater than zero.

Conversely, when the potassium concentration in the serosal medium was increased above that with which the hemibladders had been equilibrated, SCC and p.d. transiently decreased and then recovered. If the potassium concentration in the medium with which the hemibladders had first equilibrated was less than 2 mM, then increasing the serosal potassium concentration led to a steady SCC greater than that previously recorded. However, if the potassium concentration in the medium with which the hemibladders had first equilibrated was greater than 2 mM, the SCC simply recovered to its previous steady value. With potassium concentrations of 48 and 116 mM, the steady SCC was the result of a significant fall in transepithelial p.d. accompanied by an increase in tissue conductance.

The initial transient changes in SCC, when the serosal medium potassium concentration was altered, did not simply reflect alterations in net passive transepithelial diffusion of potassium as a result of changed chemical gradients across the tissue (section *v* of Results). However, when hemibladders had been incubated in media with high potassium con-

centrations, the resulting increased conductance of the tissue may have allowed a significant contribution of potassium diffusion to the initial transient of SCC when serosal potassium concentration was subsequently changed.

The results presented indicated that the initial transient alterations in SCC appeared to be only partly accounted for by changes in M→S sodium flux, in which case some other ion or ions must also be moving across the epithelial cell membranes. This problem cannot be resolved adequately without a knowledge of the changes in epithelial cell composition that accompany changes in medium potassium concentration. The transients will therefore be discussed in detail in the next paper (Robinson & Macknight, 1976*a*). Several points can, however, be made here.

Although they only partially reflected changes in M→S Na flux, the transients in SCC did however seem to depend upon the basic transporting capacity of the individual hemibladders. For example, although the initial SCC's of the hemibladders exposed to Na Ringer's ranged from 50 to 450 μA, the peak in SCC when serosal potassium was removed always represented a doubling of SCC. In addition, removal of all potassium from serosal media of 2 to 116 mM potassium resulted in similar increments of SCC over the SCC in Na Ringer's. This conclusion is supported by the result that initial partial inhibition of SCC, as a result of equilibration of hemibladders with serosal media of potassium concentration less than 2 mM, was associated with a smaller percentage increase in SCC when the serosal medium was made potassium-free. Alternatively, when the mucosal sodium concentration was progressively decreased, thereby progressively decreasing sodium transport, the peak in SCC on removal of serosal potassium also progressively decreased (Robinson & Macknight, *unpublished observations*).

The initial transient increase in SCC of toad bladders on removing serosal potassium has been almost ignored except for brief mentions by Essig (1965) and Finn *et al.* (1967), despite the observed initial stimulation of SCC and p.d. on removing potassium from the solution bathing the inner surface of frog skin (Ussing, 1965). Recently, Finn and Hutton (1974) described the transient increase in SCC on reducing serosal potassium from 2.5 to 1.0 mM. The results presented here, from experiments employing Dominican toads, are consistent with their results, from experiments with Colombian toads.

Transient decreases in SCC and p.d., on increasing serosal potassium concentration, similar to those described here, have been reported previously for serosal potassium concentrations less than about 20 mM

(Gatzy & Clarkson, 1965; Finn, 1973, 1974; Finn & Hutton, 1974). However, Sullivan, Tucker and Scherbenske (1971) observed a transient increase in SCC on increasing serosal potassium concentration. The transient depression of SCC on applying a K Ringer's serosal medium has been previously described in frog skin (Bricker, Biber & Ussing, 1963; Ussing, 1965) and in toad bladder (Pour-Hassani & Finn, 1974). The immediate depression and subsequent recovery of SCC was attributed entirely to inhibition and stimulation, respectively, of sodium transport (Pour-Hassani & Finn, 1974). This interpretation is not supported by our results.

Other workers have also referred to the effects that altering serosal potassium concentration has on the stable SCC. Thus, Mendoza (1972) and Finn (1973) have reported some increase in steady SCC after small increases in serosal potassium concentrations, and Mendoza (1972) observed that larger increases depressed the steady SCC. Effects of K Ringer's on SCC and conductance in both toad bladder (Gatzy & Clarkson, 1965; Leb, Hoshiko & Lindley, 1965; Pour-Hassani & Finn, 1974) and frog skin (Bricker *et al.*, 1963) similar to those reported here, have been observed.

The nature of the potassium dependency of transepithelial sodium transport will also be discussed in greater detail when the effects of variations in serosal medium potassium concentration on epithelial cellular composition are presented (Robinson & Macknight, 1976*a*). However, the initial peak of SCC, and stimulation of sodium transport, on removal of serosal potassium argues against any explanation for the subsequent inhibition of transport based on a direct dependency of sodium extrusion from the cells on potassium in the serosal medium. If this were true, then one would predict an immediate inhibition of SCC on removal of serosal K, for under these conditions the potassium concentration in the serosal medium surrounding the epithelial cells should have fallen at once. The fact that ouabain, a known inhibitor of the sodium pump (Schatzmann, 1953; Glynn, 1964), produces an immediate inhibition of SCC in either a K-free Na Ringer's (Robinson & Macknight, 1976*a*) or in Na Ringer's (Macknight, Civan & Leaf, 1975) supports this interpretation. Furthermore, the abilities of lithium Ringer's and choline Ringer's to reduce the extent of the inhibition of SCC by removal of serosal potassium suggests that it is not absence of serosal potassium *per se* that inhibits sodium transport by toad bladder.

The abilities of other cations to replace serosal medium potassium were of interest. It was clear that the alkali cations could only partially

replace potassium in terms of their effects on SCC, and the degree to which they accomplished this differed between the ions. For example, the inhibition of SCC associated with 3.5 mM Rb-Na Ringer's was as great as that seen with 1.5 mM K-Na Ringer's; whereas the inhibition of SCC associated with 3.5 mM Cs-Na Ringer's was as great as that seen with a 0.7 mM K-Na Ringer's. However, with 3.5 mM Li-Na Ringer's the inhibition of SCC was as great as with 3.5 mM Na-Na Ringer's, i.e. K-free Na Ringer's, as was that associated with 3.5 mM choline-Na Ringer's. Sodium cannot be considered a substitute for serosal potassium in the same way as the other alkali cations or choline used here, for sodium is already present in a high concentration in all these media. But since SCC decreased to very low levels, in K-free Na Ringer's, despite the high serosal sodium concentration, sodium must be regarded as a very poor substitute for serosal potassium.

From these results (Fig. 9) the alkali cations and choline can be ranked in order of their effectiveness in maintaining SCC when present at a concentration of 3.5 mM in the potassium-free serosal medium:

$$K > Rb > Ca > Li, \text{ choline.}$$

This order does not correspond to their relative hydrated sizes, nor to the sizes of the ions in crystals, nor to their conductivities in dilute aqueous solutions. Neither does the ranking of the alkali cations shown above correspond to any of those predicted from the coulombic sequences of the ions (Diamond, 1971). This lack of correspondence suggests that the ability of an alkali cation to replace serosal potassium may not depend simply on the ease with which the cation can diffuse across the cellular membrane. Nevertheless, these results are consistent with results which indicate that toad bladder, and frog skin, are almost as permeable to rubidium as to potassium, somewhat less permeable to caesium and relatively impermeable to lithium and sodium (Leb *et al.*, 1965; Hoshiko, 1973).

Finally, the results of these experiments show that the usual potassium concentration of 3.5 mM in Na Ringer's lies well within the physiological range. Not until medium potassium concentration is reduced below 2 mM or increased above 14 mM are there any detectable effects of variations in medium potassium on SCC or p.d. Nor, over this range of concentration, is there any detectable effect on cellular composition (Robinson & Macknight, 1976*a*).

This work was supported by the Medical Research Council of New Zealand. B.A.R. was the recipient of an M.R.C. Research Scholarship in Medical Sciences. We are grateful to Professor A. Leaf for helpful discussions during a visit to Dunedin as Harold Chaffer Lecturer and to both him and Dr. M.M. Civan for their critical reading of the manuscript.

References

Bentley, P.J. 1959. The effects of ionic changes on water transfer across the isolated urinary bladder of the toad *Bufo marinus*. *J. Endocrinol.* **18**:327

Bricker, N.S., Biber, T.U.L., Ussing, H.H. 1963. Exposure of the isolated frog skin to high potassium concentrations at the internal surface. I. Bioelectric phenomena and sodium transport. *J. Clin. Invest.* **42**:88

Diamond, J.M. 1962. The reabsorptive function of the gallbladder. *J. Physiol.* **161**:442

Diamond, J.M. 1971. Water-solute coupling and ion selectivity in epithelia. *Philos. Trans. R. Soc. London Ser. B.* **262**:141

Essig, A. 1965. Active sodium transport in toad bladder despite removal of serosal potassium. *Am. J. Physiol.* **208**:401

Essig, A., Leaf, A. 1963. The role of potassium in active transport of sodium by the toad bladder. *J. Gen. Physiol.* **46**:505

Finn, A.L. 1973. Ouabain-dependent potassium-potassium exchange in the toad bladder. *J. Membrane Biol.* **12**:301

Finn, A.L. 1974. Transepithelial potential difference in toad urinary bladder is not due to ionic diffusion. *Nature* **250**:495

Finn, A.L., Handler, J.S., Orloff, J. 1967. Active chloride transport in the isolated toad bladder. *Am. J. Physiol.* **213**:179

Finn, A.L., Hutton, S.A. 1974. The kinetics of sodium transport in the toad urinary bladder. III. The role of potassium. *J. Membrane Biol.* **17**:253

Gatzy, J.T., Clarkson, T.W. 1965. The effect of mucosal and serosal solution cations on bioelectric properties of the isolated toad bladder. *J. Gen. Physiol.* **48**:647

Glynn, I.M. 1964. The action of cardiac glycosides on ion movements. *Pharmacol. Rev.* **16**:381

Hays, R.M., Leaf, A. 1961. The problem of clinical vasopressin resistance: In vitro studies. *Ann. Intern. Med.* **54**:700

Hoshiko, T. 1973. Cation selectivities in frog skin. *In:* Transport Mechanisms in Epithelia. H.H. Ussing and N.A. Thorn, editors. p. 99. Munksgaard, Copenhagen

Koefoed-Johnsen, V., Ussing, H.H. 1958. The nature of the frog skin potential. *Acta Physiol. Scand.* **42**:298

Leb, D.E., Hoshiko, T., Lindley, B.D. 1965. Effects of alkali metal cations on the potential across toad and bullfrog urinary bladder. *J. Gen. Physiol.* **48**:527

Macknight, A.D.C., Civan, M.M., Leaf, A. 1975. Some effects of ouabain on cellular ions and water in epithelial cells of toad urinary bladder. *J. Membrane Biol.* **20**:387

Macknight, A.D.C., DiBona, D.R., Leaf, A., Civan, M.M. 1971. Measurement of the composition of epithelial cells from the toad urinary bladder. *J. Membrane Biol.* **6**:108

Mendoza, S.A. 1972. Potassium dependence of base-line and ADH-stimulated sodium transport in toad bladder. *Am. J. Physiol.* **223**:120

Pour-Hassani, H., Finn, A.L. 1974. Effect of serosal potassium-sodium substitution on sodium and potassium kinetics in toad bladder. *Fed. Proc.* **33**:216

Robinson, B.A., Macknight, A.D.C. 1976*a*. Relationships between serosal medium potassium concentration and sodium transport in toad urinary bladder. II. Effects of different medium potassium concentrations on epithelial cell composition. *J. Membrane Biol.* **26**:239

Robinson, B.A., Macknight, A.D.C. 1976*b*. Relationships between serosal medium potassium concentration and sodium transport in toad urinary bladder. III. Exchangeability of epithelial cellular potassium. *J. Membrane Biol.* **26**:269

Schatzmann, H.J. 1953. Herzglykoside als Hemmstoffe für den aktiven Kalium- und Natriumtransport durch die Erythrocytenmembran. *Helv. Physiol. Acta* **11**:346

Sullivan, L.P., Tucker, J.M., Scherbenske, M.J. 1971. Effect of furosemide on sodium transport and metabolism in toad urinary bladder. *Am. J. Physiol.* **220:**1316

Ussing, H.H. 1960. The Alkali Metal Cations in Biology. H.H. Ussing, P. Kruhoffer, J.H. Thaysen and N.A. Thorn, editors. Springer-Verlag, Berlin-Heidelberg-Göttingen

Ussing, H.H. 1965. Relationship between osmotic reactions and active sodium transport in the frog skin epithelium. *Acta Physiol. Scand.* **63:**141