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

III. Exchangeability of Epithelial Cellular Potassium

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Summary. The exchangeability of toad bladder epithelial cell potassium has been investigated. An insignificant amount of cellular potassium exchanged with mucosal medium ^{42}K. From the rate of uptake of ^{42}K into the cells from the serosal medium at least two cellular potassium pools were identified. The more rapidly exchanging pool contained about one-quarter to one-third of the cellular potassium and exchanged with a half-time of about 30 min. It was from this pool that potassium was lost from cells exposed to ouabain or to a potassium-free medium. In addition, when 3.5 mM rubidium replaced 3.5 mM potassium in sodium Ringer's the epithelial cells lost in 60 min about one-quarter of their cellular potassium in exchange for rubidium. Inhibition of transepithelial sodium transport by amiloride, 10^{-5} mM, seemed to depress the rate of potassium uptake into the more rapidly exchanging pool without affecting total cellular potassium content. However, stimulation of transepithelial sodium transport by vasopressin appeared not to affect the rate of potassium uptake. The rate of potassium uptake into this pool seemed much less than that required for a tight 1:1 coupling between transepithelial sodium transport and potassium uptake. The remaining cellular potassium exchanged at a much slower rate and even after 19 hours of incubation only 67% of cellular potassium was labelled. If this slower exchanging potassium represents a single pool, 99% of cellular potassium would be labelled only after incubation with ^{42}K for 56 hours.

The development of techniques for the determination of cellular composition in toad bladder epithelial cells has allowed measurements of cellular water, sodium, potassium and chloride, and determination of sodium exchange with both mucosal and serosal medium sodium, under a variety of experimental conditions (Macknight, DiBona, Leaf & Civan, 1971; Macknight, Leaf & Civan, 1971; Macknight, Civan & Leaf, 1975 *a*, *b*). The previous paper provided results of experiments in which cellular composition was determined in the presence of different serosal medium potas-

sium concentrations (Robinson & Macknight, 1976*b*). However, though potassium uptake and the exchangeability of potassium had been determined in whole toad hemibladders (Essig & Leaf, 1963; Finn & Nellans, 1972; Kallus & Vanatta, 1970) there are no data available about potassium exchangeability in toad bladder epithelial cells themselves. The present work therefore investigated the exchangeability of potassium between toad bladder epithelial cells and the mucosal and serosal media under a variety of conditions. There was only a very small labelling of cellular K by mucosal ^{42}K. In addition, the results demonstrate that only a fraction of cellular potassium exchanges at all rapidly with the serosal medium and that it is from this relatively rapidly exchanging pool that potassium is lost from the cells into potassium-free serosal medium or exchanged for sodium in the presence of ouabain. In addition, the results provide no evidence in favor of a rigid coupling between transepithelial sodium transport and the uptake of serosal potassium to the cells.

Materials and Methods

The media and methods used in these experiments were similar to those previously described (Macknight, DiBona, Leaf & Civan, 1971; Macknight *et al.*, 1975*a*, *b*; Robinson & Macknight, 1976*a*, *b*).

Hemibladders were either mounted in chambers, incubated, and then removed, blotted and the epithelial cells scraped off and analyzed in the usual way, or, alternatively, hemibladders or hemibladders cut in half (quarter-bladders) were incubated in beakers at room temperature in aerated media. Under these conditions the tissue is short-circuited though transepithelial sodium transport cannot be studied. However, this technique had the major advantage that tissue can be rapidly removed from the media, blotted and the epithelial cells obtained for analysis, thereby facilitating kinetic studies.

Tissue sodium, potassium and chloride and cellular water were determined as described previously. Rubidium was measured on tissue and medium samples using a Jarrell-Ash Atomic Absorption Spectrophotometer. Radioactive ^{42}K (obtained from Australian Atomic Energy Commission, Lucas Heights, New South Wales) was determined by counting tissue and medium samples in a gamma scintillation spectrometer (Packard Instrument Company, Inc.). Cellular potassium equilibrated with medium ^{42}K was calculated in a manner identical to the calculation of cellular sodium equilibrated with medium ^{24}Na (Macknight *et al.*, 1975*a*) based upon the assumption that the specific activities of ^{42}K in cells and media were identical.

Because the medium potassium concentration is so low compared to the cellular potassium, in some of the kinetic experiments the extracellular space was not measured directly. Instead, all values were corrected assuming a tissue water content of 6 kg/kg dry wt and an extracellular space (mucosal and serosal) of 35 per cent tissue water. This correction, which only affected values obtained in the very first minutes of incubation, was designed to remove from the results any contribution of initial equilibration of ^{42}K in the tissue extracellular fluid. Though the results are presented in the appropriate figures with this correction, analysis of the uncorrected data yields the same conclusions.

Results

Equilibration of Mucosal Medium ^{42}K with Cellular Potassium

Hemibladders, mounted in chambers and bathed on both surfaces with sodium Ringer's, were short-circuited and then exposed on their mucosal surface alone to ^{42}K in sodium Ringer's, while sodium Ringer's without radioactive potassium bathed the serosal surface. After 60 min, when corrected for ^{42}K in the mucosal inulin space, only $1.5 \pm 0.3\%$ (6.3 mmole/kg dry wt of 438 mmole cellular potassium/kg dry wt, $n=7$) had equilibrated with mucosal ^{42}K. Of this, more than 50% was removed by a rapid washing of the mucosal surfaces of the hemibladders just prior to their removal for scraping and analysis, and therefore may not be truly intracellular. These results show that potassium exchange across the mucosal cellular membrane can make only a negligible contribution to cellular potassium exchange. This observation, consistent with that reported by others (Kallus & Vannatta, 1970; Gatzy, 1971; Finn & Nellans, 1972), justifies the technique of exposing hemibladders in beakers to ^{42}K used in kinetic experiments.

Equilibration of Serosal Medium ^{42}K with Cellular Potassium

Since there was a negligible exchange of mucosal medium ^{42}K with cellular potassium, any exchange found in hemibladders exposed on both surfaces to ^{42}K must reflect potassium movement across the serosal cellular membrane.

An initial experiment indicated that even an 8 hr exposure to ^{42}K in Na Ringer's did not result in complete labelling of all cellular potassium with ^{42}K, only 60% of the cellular potassium having equilibrated with ^{42}K ($n=6$). (In the same time 40% of the subepithelial tissue potassium had equilibrated with ^{42}K.)

An experiment was therefore performed to examine more completely the exchangeability of cellular potassium. Quarter-bladders from 16 toads were incubated 60 min in sodium Ringer's. They were then transferred to fresh sodium Ringer's containing ^{42}K. At timed intervals individual quarter-bladders were removed, blotted, scraped and the epithelial cells collected and analyzed for cellular potassium and ^{42}K. In Fig. 1 the percentage of cellular potassium which had equilibrated with ^{42}K (ordinate) is plotted against time of exposure to ^{42}K (abscissa). It can

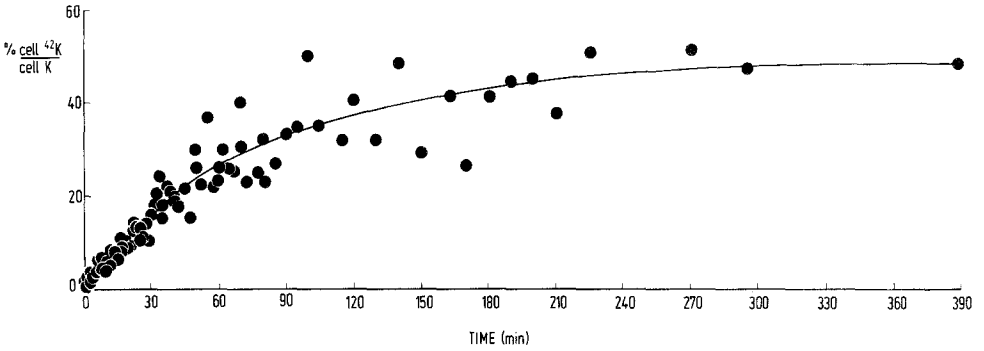

Fig. 1. Labelling of cellular potassium by medium ^{42}K with time. Quarter-bladders equilibrated with Na Ringer's in a beaker were all transferred to Na Ringer's containing ^{42}K. Quarter-bladders were removed from the medium after various times, blotted, and the epithelial cells scraped off for analysis. The percentage of cellular potassium labelled by medium ^{42}K is shown on the ordinate, with time on the abscissa

be seen that there was an initial relatively rapid labelling of cellular potassium followed by a much slower labelling. Even after 390 min, only 54% of cellular potassium had equilibrated with ^{42}K. Over this period of 390 min, cellular potassium content was constant, averaging 422 ± 4 mmoles/kg dry wt ($n=78$). One quarter-bladder was incubated for 19 hr. Even after this time only 67% of cellular potassium was labelled and cellular potassium had fallen to 340 mmoles/kg dry wt.

The results illustrated in Fig. 1 suggested that there was a fraction of cellular potassium which exchanged quite rapidly with medium potassium, and another fraction which exchanged much more slowly.

To enable analysis of these results, $\log_e$ (100-% labelled) was plotted against time and this graph is shown in Fig. 2. The slope of the line beyond 80 min was taken to represent the rate of labelling of a slowly exchangeable compartment. The correlation coefficient for the points about this line was 0.69 ($n=21$, $p<0.001$). The values for the first 40 min of incubation were then corrected for the contribution of the slow compartment and replotted. The regression line drawn through these points showed a correlation coefficient of 0.87 ($n=27$, $p<0.001$). Thus it proved possible to identify at least two cellular K compartments, the more rapidly labelled of which comprised 124 mmole/kg dry wt or 29% of cellular potassium. The second, much more slowly exchanging compartment was never completely labelled in these experiments but from the slope of the regression line it can be calculated that 99% of potassium within the compartment would be labelled after 56 hr of incubation.

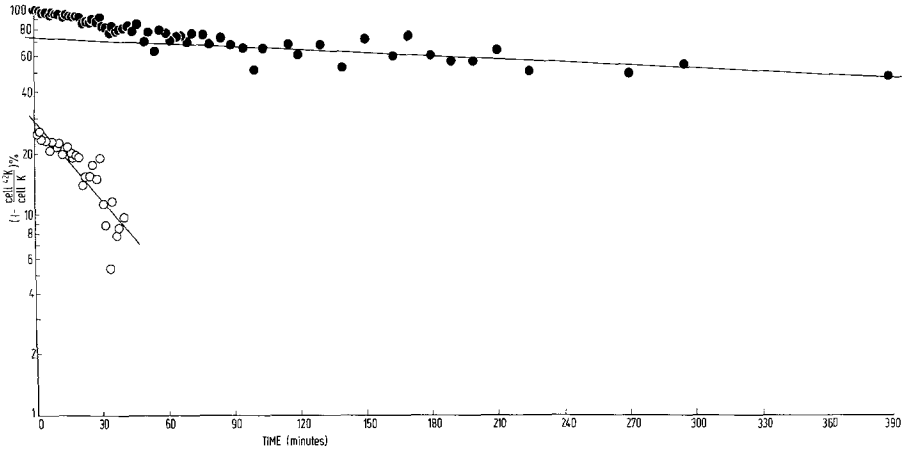

Fig. 2. Labelling of cellular potassium by medium ^{42}K with time. The ordinate shows (1-% cellular K labelled), on a log scale, while the abscissa shows time. ● represent data from Fig. 1. The regression line for all data for 80 min and longer is drawn ($y=e^{-0.0013t}+e^{4.30}$, $r=0.69$, $p<0.001$, $n=21$). This regression line was subtracted from data for 0–40 min (○), and a second line drawn ($y=e^{-0.0264t}+e^{3.31}$, $r=0.86$, $p<0.001$, $n=39$)

Effects of Changes in Transepithelial Sodium Transport on ^{42}K Uptake by Epithelial Cells

It was important to determine whether the faster exchanging cellular potassium compartment which had been identified was in any way affected by variations in transepithelial sodium transport. That some positive relationship between the labelling of the faster exchanging cellular potassium pool and transepithelial sodium transport did exist was suggested from the positive correlation illustrated in Fig. 3, between the percentage labelling of cellular potassium in relation to SCC in hemibladders bathed on both surfaces with sodium Ringer's and incubated 60 min in chambers ($r=0.63$, $n=30$, $p<0.001$).

However, a more direct examination, of this relationship was performed in an experiment in which quarter-bladders from the same toads were incubated with ^{42}K either in sodium Ringer's, sodium Ringer's containing 10^{-5} M amiloride to inhibit sodium transport, or sodium Ringer's containing vasopressin (100 mU/ml) to stimulate sodium transport, for periods up to 80 min (Fig. 4). Tissues were exposed to amiloride or to vasopressin for 15 min before transfer to the medium containing the agent and ^{42}K. This ensured that the effects of the agents were

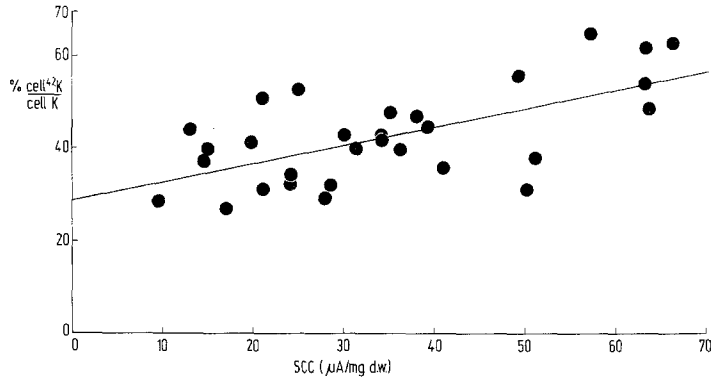

Fig. 3. Cellular uptake of ^{42}K in 60 min and SCC of toad bladder. Short-circuited hemibladders, mounted in chambers and equilibrated in Na Ringer's, were bathed by Na Ringer's + ^{14}C-inulin mucosal medium, Na Ringer's + ^{42}K + ^{3}H-inulin serosal medium for 60 min. Hemibladders were then taken from the chambers, blotted, and the epithelial cells scraped off and analyzed. This graph illustrates the percentage of cellular potassium labelled by serosal medium ^{42}K (ordinate) and the steady SCC (abscissa) in the 60-min incubation period. The SCC is expressed as μA/mg dry wt of epithelial cells. The regression line is shown

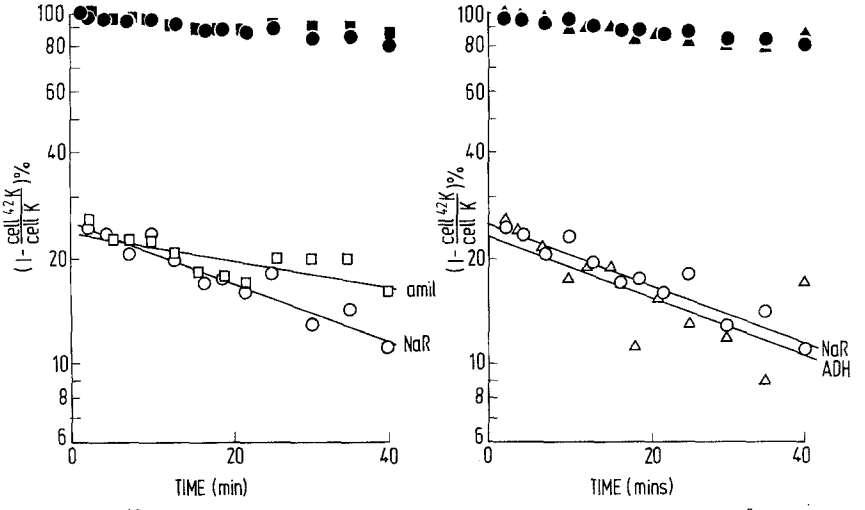

Fig. 4. Cellular ^{42}K uptake with time of incubation in Na Ringer's, with 10^{-5} M amiloride or with ADH. Quarter-bladders were incubated in beakers in Na Ringer's or Na Ringer's + 10^{-5} M amiloride for 60 min, or in Na Ringer's + 100 mU/ml ADH for 10 min. They were then transferred to Na Ringer's + ^{42}K (●), Na Ringer's + 10^{-5} M amiloride + ^{42}K (■) or Na Ringer's + 100 mU/ml ADH + ^{42}K (▲), respectively. Quarter-bladders were removed from each medium at various times between 0 and 80 min, and the epithelial cells were scraped off for analysis. The graph shows (1-% cellular K labelled) on a log scale as the ordinate, with time the abscissa as in Fig. 2. Subtraction of the regression line for data beyond 80 min (Fig. 2) resulted in points shown by open symbols. The regression lines are: Na Ringer's, $y=e^{-0.0187t}+e^{3.21}$, $r=0.94$, $p<0.001$, $n=12$. Na Ringer's $+10^{-5}$ M amiloride, $y=e^{-0.0085t}+e^{3.15}$, $r=0.75$, $p<0.005$, $n=12$; Na Ringer's+ADH, $y=e^{-0.0191t}+e^{3.13}$, $r=0.74$, $p<0.01$, $n=12$

established before exposure to ^{42}K. Correction as before for the slow pool resulted in the regression lines shown in Fig. 4. The assumption underlying this experimental approach was that of the two pools which had been identified, only the more rapidly exchanging pool could be related to transepithelial sodium transport. It was therefore accepted that neither agent would affect the slower pool which was assumed to have the same characteristics as those shown in Fig. 2. (Experiments in chambers with ^{42}K which showed no difference in the percentage of cellular K labelled with ^{42}K after 60 min with or without vasopressin support this assumption and are in agreement with the observations shown in Fig. 4 for vasopressin.) Statistical analysis was performed to determine whether or not the regression lines for the fast pool were parallel, and if parallel, identical. The slope of the regression line for ^{42}K uptake in the presence of amiloride was less steep than that for ^{42}K uptake in sodium Ringer's ($p < 0.005$) suggesting that cells exchanged potassium less rapidly with transepithelial sodium transport inhibited. However, stimulation of sodium transport by ADH did not alter the slope of the line ($p > 0.30$). In all these experiments mean cellular potassium contents were not significantly different, nor were there any significant changes in cellular potassium content with duration of incubation. Therefore, it is concluded that cellular potassium remained in a steady state throughout this experiment.

Effects of Different Medium Potassium Concentrations on the Rate of ^{42}K Uptake by Epithelial Cells

The preceding paper (Robinson & Macknight, 1976*b*) presents results which demonstrated that when medium potassium concentration is either reduced below about 2 mM or is substantially increased above the normal 3.5 mM, cellular potassium is measureably altered. However, increasing medium potassium concentration even fourfold, from 3.5 to 14 mM did not affect the cellular potassium after 60 min of incubation. It was however possible that these smaller alterations in medium potassium concentration might affect the rate at which potassium exchanged across the serosal cellular membrane.

Experiments were therefore performed with quarter-bladders incubated in beakers containing either 2.0 mM or 7.8 mM potassium in sodium Ringer's. Neither of these medium potassium concentrations affects the steady SCC (Robinson & Macknight, 1976*a*). After 60 min the quarter-

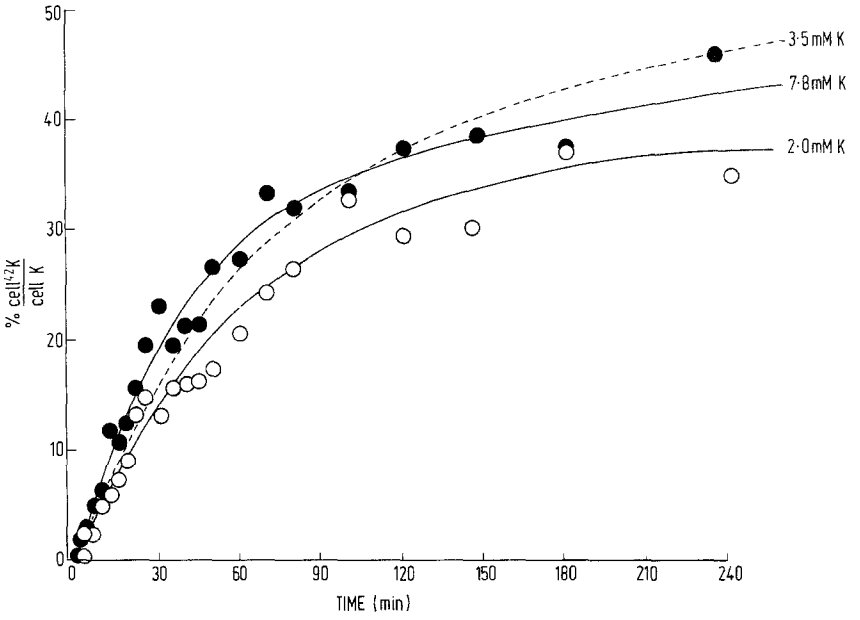

Fig. 5. Cellular ^{42}K uptake in media of 2.0 and 7.8 mM potassium. Quarter-bladders equilibrated 60 min in beakers in either 2.0 (○) or 7.8 (●) mM K-Na Ringer's (total Na+K in media constant), were transferred to beakers of identical media but containing ^{42}K. Quarter-bladders were removed, blotted, and the epithelial cells scraped off for analysis at various times up to 240 min. Lines of best fit are drawn; the dashed line is that for 3.5 mM K, i.e., Na Ringer's, from Fig. 1

bladders were transferred to fresh media of the same composition in which they had equilibrated but containing ^{42}K. The epithelial cells were obtained for analysis at timed intervals between 0 and 240 min incubation in the media containing ^{42}K. The results obtained are illustrated in Fig. 5, which also contains the best fit line, but not the individual points, for ^{42}K uptake in sodium Ringer's.

The results were analyzed in the same way as before, the regression line for values after 80-min incubation being calculated and the values for the first 40 min of incubation corrected for the contribution to them of the slower labelling compartment. The slopes of the regression lines of the faster exchanging compartment for medium potassium concentrations of 2 mM, 3.5 mM and 7.8 mM were not statistically significantly different (2 mM compared with 3.5 mM, $p > 0.50$, 3.5 mM compared with 7.8 mM, $p > 0.05$). In each of the media the cellular potassium content remained constant throughout the experiment. However, though the potassium contents of cells incubated with 2 mM and 3.5 mM medium potassium concentrations

did not differ significantly ($\Delta = -4 \pm 10$ mmole/kg dry wt, $p > 0.70$), cells incubated with a medium potassium concentration of 7.8 mM contained slightly more potassium (444 ± 10 mmole/kg dry wt, $n = 22$, $\Delta = 22 \pm 10$ mmole/kg dry wt, $p < 0.05$) when compared with cells incubated with 3.5 mM medium potassium concentration.

Labelling of Cellular Potassium with Low Medium Potassium Concentration

The previous section presents results which show that a reduction in medium potassium concentration from 3.5 to 2 mM detectably affects neither the rate at which cellular potassium is labelled nor the cellular potassium content. Neither does a reduction in medium potassium of this magnitude affect the transepithelial p.d. or SCC (Robinson & Macknight, 1976*a*). However, more substantial decreases in medium potassium concentration are accompanied by decreased transepithelial p.d. and SCC (Bentley, 1960; Hays & Leaf, 1961; Essig & Leaf, 1963; Davies, Martin & Sharp, 1968; Mendoza, 1972; Robinson & Macknight, 1976*a*), and loss of cellular potassium, partly in exchange for serosal sodium, partly with chloride and water (Robinson & Macknight, 1976*b*). It was therefore of importance to examine the effects of a low medium potassium concentration on cellular labelling by ^{42}K. Since ^{42}K had to be present

Table 1. Exchange of cellular potassium with serosal ^{42}K in 0.5 mM K serosal medium

Serosal solution	Cell water (kg/kg dry wt)	Cell ions (mmole/kg dry wt)			
		Na	K	^{42}K	Cl
Na Ringer's	3.83	267	429	181	286
0.5 mM K-Na Ringer's	3.50	302	349	69	248
$\Delta \pm$ SE	-0.34 ± 0.08	35 ± 18	-80 ± 11	-112 ± 18	-37 ± 17
p	< 0.01	> 0.10	< 0.001	< 0.001	> 0.05
$n = 6$					

SCC after 60 min in 0.5 mM K-Na Ringer's serosa: $50 \pm 10\%$ of previous SCC in Na Ringer's.

Paired hemibladders were mounted in chambers, bathed with Na Ringer's and continously short-circuited. The mucosal and serosal media were then drained. The mucosal chambers were all filled with Na Ringer's + ^{14}C-inulin. One hemibladder of each pair was bathed by a 0.5 mM K-Na Ringer's serosal medium, the other by Na Ringer's. All serosal media contained ^{3}H-inulin and ^{42}K. After 60 min, the hemibladders were taken from the chambers, blotted, and the epithelial cells scraped off for analysis.

in the serosal medium it was not possible to perform these experiments with a potassium-free serosal medium. Instead, paired hemibladders were exposed, in chambers, to sodium Ringer's or sodium Ringer's containing only 0.5 mM potassium on their serosal surface. Both serosal media contained ^{42}K at the same specific activity. Hemibladders were continuously short-circuited under these conditions for 60 min; hemibladders were then removed from the chambers, blotted, and the epithelial cells scraped off for analysis. The results of these experiments are contained in Table 1.

As expected, the decrease in medium potassium concentration resulted in loss of potassium partly in exchange for sodium, partly with chloride and water. The potassium lost had come from the more rapidly labelled cellular potassium pool for the loss of cellular potassium and the decrease in cellular potassium equilibrating with ^{42}K were of the same magnitude and did not differ statistically ($\Delta = 32 \pm 21$ mmole/kg dry wt, $p > 0.10$).

Labelling of Potassium in Epithelial Cells Exposed to Ouabain

The cardiac glycoside ouabain in the serosal medium inhibits transepithelial sodium transport by toad bladder (Herrera, 1966) and results in a loss of cellular potassium, partly in exchange for serosal sodium, partly in exchange for mucosal sodium (Macknight *et al.*, 1975*b*). The magnitude of the potassium loss after 60 min of incubation is variable even when the cells are exposed to a concentration of ouabain of 10^{-2} M, more than sufficient to produce a complete inhibition of transepithelial sodium transport. However, the observation that the epithelial cellular potassium consisted of at least two distinct pools suggested that the more rapidly exchanging of these pools should be the source of the potassium lost after exposure of the cells to ouabain. This was confirmed in experiments using paired hemibladders mounted in chambers, the results of which are shown in Table 2. As usual, the presence of ouabain, though it did not affect cellular water or chloride contents, resulted in a loss of cellular potassium in exchange for medium sodium. At the end of 60 min of incubation only 38 mmoles of cellular potassium/kg dry wt had equilibrated with medium potassium in the presence of ouabain, whereas in the controls not exposed to ouabain 185 mmoles/kg dry wt had been labelled. The difference of 146 ± 11 mmole/kg dry wt between these values was not significantly different from the quantity of potassium lost from the cells exposed to ouabain ($\Delta = 26 \pm 15$ mmole/kg dry wt, $p > 0.10$). These results therefore establish that the potassium

Table 2. Effect of ouabain, 10^{-2} M, on cellular ^{42}K uptake

Serosal solution	Cell water (kg/kg dry wt)	Cell ions (mmole/kg dry wt)			
		Na	K	^{42}K	Cl
Na Ringer's	3.58	255	394	185	275
Na Ringer's + ouabain	3.65	405	274	38	279
$\Delta \pm$ SE	0.07 ± 0.21	150 ± 19	−120 ± 15	−146 ± 11	4 ± 23
p	>0.70	<0.001	<0.001	<0.001	>0.80
$n=8$					

Paired hemibladders were mounted in chambers, bathed with Na Ringer's, and continuously short-circuited. The mucosal and serosal media were then drained. The mucosal chambers were all filled with Na Ringer's + ^{14}C-inulin. One hemibladder of each pair was bathed by Na Ringer's + 10^{-2} M ouabain serosal medium, the other by Na Ringer's. All serosal media contained ^{42}K and ^{3}H-inulin. After 60 min, the hemibladders were taken from the chambers, blotted, and the epithelial cells scraped off for analysis.

lost from toad bladder epithelial cells exposed to ouabain had come from the rapidly exchanging cellular potassium pool and suggest that by 60 min this pool had been largely depleted of potassium.

Exchangeability of Epithelial Cell Potassium for Medium Rubidium

Results presented in a previous paper (Robinson & Macknight, 1976*a*) showed that complete replacement of medium potassium by rubidium at the same concentration, 3.5 mM, resulted in very little disturbance in transepithelial p.d. or SCC either immediately when the solutions were changed or in the new steady levels subsequently achieved. This suggested that rubidium might replace cellular potassium and a series of experiments, the results of which are presented in Table 3, were performed to examine this possibility. There was a substantial loss of cellular potassium of the same order as would occur from cells exposed to a potassium-free Ringer's solution (Table 1, Robinson & Macknight, 1976*b*). However, instead of losses of water and chloride and some increases in cellular sodium, the cells gained a quantity of rubidium similar to the potassium lost and there were no changes in cellular water, sodium, sodium equilibrated with mucosal medium sodium, or chloride. Thus, there was a one-for-one exchange of cellular potassium for medium rubidium, these exchanges presumably occurring within the faster of the two cellular potassium pools which have been characterized in this paper.

Table 3. Epithelial cell composition when serosal potassium was replaced by rubidium

Serosal solution	Cell water (kg/kg dry wt)	Cell ions (mmole/kg dry wt)				
		Na	^{24}Na	K	Rb	Cl
Na Ringer's	3.20	221	48	419	0	269
3.5 mM Rb-Na Ringer's	3.24	219	44	312	117	256
$\Delta \pm$ SE	0.04 ± 0.20	−2 ± 24	−4 ± 3	−107 ± 7	117 ± 12	−13 ± 29
p	>0.80	>0.90	>0.20	<0.001	<0.001	>0.60
$n=8$						

Paired hemibladders were mounted in chambers, bathed with Na Ringer's, and continuously short-circuited. The mucosal and serosal media were then drained and replaced with fresh Na Ringer's; ^{24}Na and ^{14}C-inulin were included in the mucosal solution. After 60 min, one hemibladder of each pair was bathed by a 3.5 mM Rb-Na Ringer's (containing no K) serosal medium, the other by Na Ringer's. Both serosal media contained ^{3}H-inulin. Sixty minutes later, the hemibladders were taken from the chambers, blotted, and the epithelial cells scraped off for analysis.

Discussion

The results presented in this paper provide evidence for the existence of at least two cellular potassium pools in toad bladder epithelial cells. One, comprising about one-quarter to one-third of the cellular potassium, exchanges relatively rapidly with potassium in the serosal medium (the half-time for labelling of this pool is about 30 min); the other exchanges much more slowly and it was not possible, even after incubation for 19 hr, to label all cellular potassium. Only an insignificant exchange of cellular potassium with mucosal medium potassium was detected.

A major disadvantage of analysis of the kinetic data presented in this paper lies in the inability to distinguish with certainty the precise number of cellular potassium compartments. Though there certainly seem to be two, there may in fact be more. It might be possible with a more sophisticated analysis to break down the data to yield more compartments. However, the inevitable scatter resulting from the necessary pooling of results from cells obtained from different hemibladders would certainly complicate such an attempt. Considerably less scatter is of course obtained in washout experiments such as those of Finn and Nellans (1972), where radioactivity lost from a whole hemibladder preincubated with ^{42}K, is recorded. However, the interpretation of the results obtained in washout studies is made difficult by the contribution

of radioactivity from subepithelial supporting tissues. Our preliminary observations suggest that cellular potassium in these subepithelial tissues also, may not all occupy a single cellular compartment. Unfortunately, no experimental technique is presently available which would allow washout experiments to be performed on viable epithelial cells alone.

It is therefore difficult to resolve the discrepancies between the results presented in this paper from analysis of ^{42}K uptake by epithelial cells alone and those obtained by Finn and Nellans (1972) and Finn (1973) from washout experiments performed using preloaded whole hemibladders. However, using the data presented by Macknight *et al.* (1975*a*) which enable expression of the composition of the epithelial cells alone in terms of the whole hemibladder dry weight it can be calculated that the total potassium in the epithelial cells is 4.5 μmole/100 mg tissue dry wt. Of this about one-third, or 1.5 μmole/100 mg tissue dry wt, is potassium in the more rapidly exchanging potassium pool. The fast pool found by Finn and Nellans (1972) contained between 1.74 and 3.46 μmole potassium/100 mg dry wt. However, though the sizes of these pools are somewhat similar, the half-times of the pools are very different: about 30 min in the present experiments, only 2.4 min in the experiments of Finn and Nellans (1972). It does not seem possible that our technique would have failed to reveal a labelling of between one- and two-thirds of the potassium within the epithelial cell with a half-time of 2.4 min and therefore it is concluded that a major part of this rapid pool described by Finn and Nellans (1972) is contributed by a washout of ^{42}K from subepithelial cells. The size of the slow pool described by Finn and Nellans (1972), ranging as it does between 7.93 and 12.20 μmoles/100 mg dry wt shows that it cannot represent solely a potassium pool within the epithelial cells. In addition, the failure of the washout technique to demonstrate any loss of potassium from either the fast or slow potassium pools in tissues exposed to ouabain (Finn, 1973), must raise doubt as to the contribution of epithelial cellular potassium to either of these potassium pools. It has been consistently reported that both in isolated sheets of epithelial cells (Lipton & Edelman, 1971; Macknight, DiBona, Leaf & Civan, 1971; Handler, Preston & Orloff, 1972) and in epithelial cells scraped from hemibladders which have been incubated in chambers (Macknight *et al.*, 1975*b*; Robinson & Macknight, 1976*b*), ouabain produces a loss of about one-third of cellular potassium in exchange for medium sodium. The present paper demonstrates that this loss occurs from the faster of the two potassium pools which we have identified.

Relationships Between Cellular Potassium Pools and Transepithelial Sodium Transport

The relationship in toad bladder between the active extrusion of sodium from the cell across the serosal membrane and the uptake of potassium to the cell remains controversial. The initial hypothesis put forward for frog skin by Koefoed-Johnsen and Ussing (1958) of a tight 1:1 coupling of the movement of these ions by an electrically neutral mechanism, though it has received some support in toad bladder (Gatzy & Clarkson, 1965; Leb, Hoshiko & Lindley, 1965; Finn & Nellans, 1972), is now thought to be less likely than an electrogenic sodium pump as first proposed by Frazier and Leaf (1963). This is true both in toad bladder (Finn, 1974) and frog skin (Biber, Aceves & Mandel, 1972; Ussing, Erlij & Lassen, 1974).

The present results lend no support to the concept of a tight coupling between sodium transport and potassium uptake. From Fig. 2 it can be seen that the more rapidly exchanging potassium pool comprises 27.5% of a cellular potassium which had a mean of 417 mmoles/kg dry wt; that is, the mean of the cellular potassium in the rapidly exchanging potassium pool was 115 mmoles/kg dry wt. From the size of the pool and the slope of the line (rate coefficient, $k=0.0264$) the unidirectional flux of the rapidly exchanging potassium across the membrane can be calculated to be 3.04 mmoles/min/kg dry wt. In this series of experiments the transepithelial transport of sodium was not measured. However, Macknight *et al.* (1975*b*) have provided data which show that in 45 hemibladders the SCC ranged between 14 and 193 μA/mg epithelial cell dry wt with a mean of 58 μA/mg dry wt. The transepithelial transport of sodium in the hemibladders used in the present experiments is unlikely to have been appreciably outside of this range. This range represents a net transepithelial sodium transport of between 8.7 and 120.0 mmoles/min/kg dry wt with a mean of 36.1 mmoles/min/kg dry wt. Since even the lowest SCC reflects a movement of sodium which is about three times greater than the rate of uptake of potassium into the more rapidly exchanging cellular potassium pool, it would seem most unlikely that there is a tight 1:1 coupling of sodium extrusion to potassium uptake across the serosal membrane of the epithelial cells. To the extent that any serosal sodium enters the transporting cells and is extruded by a sodium pump in exchange for serosal potassium, these calculations will underestimate the ratio of sodium extruded to potassium taken up by the cell.

The more slowly exchanging of the cellular potassium pools seems unlikely to be involved in transepithelial sodium transport. If it were, since the exchange of potassium is so slow, it could not possibly provide a tight linkage between sodium extrusion and potassium uptake.

The present results therefore favor electrogenic sodium transport rather than electrically neutral coupled sodium-potassium exchange, a conclusion consistent with the recent evidence provided by Finn (1974), which suggests that the transepithelial potential difference in toad urinary bladder is not due simply to ionic diffusion.

The relationship between transepithelial sodium transport and cellular uptake of serosal potassium as a consequence of the p.d. generated across the serosal cellular membrane by sodium extrusion is presumably reflected in Fig. 3, which demonstrates a dependency of the rate of potassium uptake into epithelial cells on SCC. Electrogenic sodium transport may also explain the effect of amiloride, 10^{-5} M, which seemed to slow the uptake of ^{42}K into the more rapidly labelled pool significantly (Fig. 4). Bentley (1968) has shown that, at this concentration, in the mucosal medium amiloride depresses SCC to 17% of the control value within 10 min. This concentration of amiloride was without effect on SCC when present only in the serosal medium.

However, though inhibition of sodium transport by amiloride was associated with a decrease in the rate of labelling of the faster cellular potassium pool, stimulation of sodium transport by vasopressin at a concentration (100 mU/ml) which would normally increase SCC by 100% or more did not alter the rate of ^{42}K labelling (Fig. 4). The explanation for this observation remains conjectural. For example, it may be, if potassium enters the cells by a carrier-mediated process rather than by simple passive diffusion, that with normal rates of sodium transport the rate of turnover of potassium carriers is already maximal and that further stimulation of sodium transport cannot increase the rate of potassium entry to the cells, leading instead to an increased p.d. across the tissue.

In contrast to the present results, Finn and Nellans (1972) reported a significant increase in the size of their rapidly exchanging potassium pool after vasopressin. The magnitude of their increase, from 1.74 to 4.05 μmoles/100 mg dry wt, was such that had such an increase occurred only in the epithelial cells, then in our experiments either the total cellular potassium would have increased from about 422 mmoles/kg dry wt to about 639 mmoles/kg dry wt, or, alternatively, all the cellular potassium would have been labelled as part of the faster of the two potassium

pools. The first of these two possibilities may be rejected. Not only was cellular potassium unchanged in the present experiments after vasopressin but also a large number of analyses, both of isolated epithelial cells incubated with vasopressin and of epithelial cells scraped from hemibladders which have been incubated in chambers, have failed to reveal any significant increases in total cellular potassium after exposure of tissue to vasopressin (Lipton & Edelman, 1971; Macknight, Leaf & Civan, 1971; Handler, Preston & Orloff, 1972). The second possibility must also be rejected for we could not have failed to detect an increase in pool size which would have labelled all rather than about one-third of cellular potassium with a half-time of 30 min or so. It is therefore difficult at the present time to resolve this difference between our own results with vasopressin and those reported by Finn and Nellans (1972).

The Maintenance of Cellular Potassium

Though the present results show some dependency of the rate of potassium uptake to the cells across the serosal membrane on transepithelial sodium transport (Fig. 3), it seems clear that the maintenance of a normal cellular potassium content does not require transepithelial sodium transport. Neither replacement of mucosal medium sodium by choline, nor the abolition of sodium transport by amiloride, 10^{-4} M, in the mucosal medium, is associated with any change in cellular potassium content or concentration (Macknight *et al.*, 1975*a*). Though changes in the potassium content of the faster cellular potassium pool which represents only about one-third of the cellular potassium might be masked to some extent when only total cellular potassium is determined, the virtual identity of the values presented in Table 2 of Macknight *et al.* (1975*a*), makes this unlikely. Therefore, the slower uptake of potassium after exposure of epithelial cells to amiloride must be accompanied by a slower diffusion of potassium from the cells back to the serosal medium; that is, the turnover of potassium through the pool must have been slowed.

In contrast to the lack of effect of inhibition of transepithelial sodium transport on cellular potassium when such inhibition is produced by removal of mucosal sodium or by amiloride in the mucosal medium, ouabain in the serosal medium both blocks transepithelial sodium transport and decreases cellular potassium content and concentration. This effect on cellular potassium cannot therefore simply be the result of a decreased transepithelial p.d. Indeed, the similarities in cellular potas-

sium between open- and short-circuited hemibladders in sodium Ringer's and in potassium-free Ringer's (Robinson & Macknight, 1976*b*) support this conclusion that the magnitude of the transepithelial p.d. is not of prime importance in determining the cellular potassium. Since ouabain blocks both transepithelial sodium transport and potassium accumulation and is thought to inhibit a membrane-bound Na-K, ATPase (Skou, 1965), it is possible that this mechanism is normally involved in sodium extrusion and potassium uptake but that when entry of mucosal sodium to the cells is inhibited, cellular potassium is exchanged for serosal medium potassium.

Further work is required to clarify the nature of potassium accumulation by these cells.

In conclusion, the present experiments measuring ^{42}K uptake have identified at least two potassium pools in toad bladder epithelial cells. The claim that the more rapidly exchanging compartment identified in this way makes up about one-quarter to one-third of the cellular potassium and exchanges with a half-time of about 30 min receives striking confirmation from the observations that about one-quarter of the cellular potassium is lost when cells are exposed to a potassium-free medium (Robinson & Macknight, 1976*b*) or to ouabain (Macknight *et al.*, 1975*b*; Robinson & Macknight, 1976*b*) and that cells exchange about one-quarter of their cellular potassium for medium rubidium; in all cases with a time course similar to that observed with ^{42}K labelling of the faster potassium compartment. In addition, the potassium lost from cells exposed to a potassium-free medium or to ouabain has come from this rapidly exchanging potassium pool (Tables 1 and 2).

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