Effects of Alkali Metal Cations on the Potential across Toad and Bullfrog Urinary Bladder

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ABSTRACT Isolated urinary bladders of the bullfrog (*R. catesbeiana*) and the toad (*B. marinus*) were mounted in an Ussing chamber. Potential differences up to 114 mv were observed in bullfrog bladder when the mucosal surface was bathed in dilute Na_2SO_4 and the serosal surface in sulfate Ringer's. In experiments with bullfrogs, K was used to replace Na in the mucosal solution and Na was used for K in the serosal solutions. The selectivity was judged in terms of the relative effectiveness of the replacement cation in maintaining the bladder potential. In experiments with toads, K and Rb were equally poor replacements for Na at the mucosal border, while Rb was a good replacement for K at the serosal border. Li in the mucosal solution appeared to depress the potential in part irreversibly. At the serosal border, Li was a partially effective substitute for K, more so than was Na. However, both were poor replacements compared to Rb. The mucosal surface of the urinary bladder of both frog and toad appears to be Na-selective and the serosal surface appears to be K-selective, consistent with the Koefoed-Johnsen–Ussing model for frog skin.

INTRODUCTION

Koefoed-Johnsen and Ussing (1) reported that the isolated frog skin in the presence of a non-penetrating anion behaves as if the outside surface is selectively permeable to Na, while the inside surface is selectively permeable to K. Lindley and Hoshiko (2) extended these observations by studying the behavior of the skin potential when other alkali metal cations were substituted for Na at the outside border and for K at the inside border. They analyzed their results in terms of Goldman's constant field equation (3). An empirically fitted parameter α was interpreted to be the ratio of the "permeability co-

efficients," P, of the constant field equation and was used to compare the selectivity among the alkali metal ions.[1]

No data on the sensitivity of the potential to alkali metal cations were available for the urinary bladder. Frazier and Leaf state that they were unable to duplicate the Koefoed-Johnsen–Ussing experiment in toad bladder (5). From micropuncture studies, Frazier and Leaf felt that the observed potential across the serosal border of the toad bladder was higher than could be attributed to a K diffusion potential.

In the present study of the urinary bladder of bullfrog and toad, modification of the apparatus used for the frog skin made it possible to study the Na sensitivity of the serosal border. The relationship of the potential difference across the isolated urinary bladder to changes in the cation composition of the mucosal and serosal bathing solutions was studied.

METHODS

Initial attempts to duplicate the frog skin experiments in bladders failed, because the fragile bladder preparation became injured when the bathing solutions were changed. To prevent mechanical trauma, the bladder was supported between two nylon net screens glued to plastic rings. When the bladder was mounted in the chamber, the screens acted to prevent damage to the bladder due to hydrostatic pressure differences which occurred when changing solutions. A plastic chamber, similar to the type described by Ussing and Zerahn (6), was used. The exposed surface area of the bladder was 2 cm^2. Leaks at the face of each half of the chamber were sealed with petroleum jelly. The chambers were attached to reservoirs, and the solutions in each half simultaneously aerated and stirred with washed compressed atmospheric air.

The potential across the bladder was monitored through 3 M KCl-agar bridges connected to calomel half-cells. A Philbrick P-2 differential operational amplifier functioning as an electrometer isolation amplifier, and a Leeds-Northrup speedomax G strip chart recorder were used to record the potential. Each bladder potential was monitored once every 30 seconds by switching from chamber to chamber with the stepping switch of the recorder, so that only one P-2 was required for up to six chambers.

The reagents used were $Li_2SO_4 \cdot H_2O$ (Baker analyzed), Na_2SO_4 (anhydrous) and K_2SO_4 (Fisher certified), Rb_2SO_4 (Fairmount Chemical), tris (hydroxymethyl) aminomethane (Sigma 7–9), and calcium gluconate (Abbott). Whenever possible, solution compositions were checked using a Baird flame photometer with an Li internal standard. A Beckman Na-sensitive glass electrode (78178-V) served as an additional check of Na concentration. Total osmolality was determined with a Fiske osmometer. The initial control solutions for all experiments are shown in Table I. Experimental

[1] Lindley (4) subsequently has designated the empirically fitted parameter β in order to preserve the form of the equation under wider conditions, reserving α for the true selectivity parameter. Under certain conditions β will approach α_{ba}, the ratio P_b to P_a. One particular case of interest is where the anion; *e.g.*, SO_4, is non-penetrating.

solutions were made by modification of the Na or K concentration only. For example, in 20 meq/liter K solution, the 20 meq/liter of Na was replaced by K, but all other constituents and the pH were identical with those of the control mucosal solution. Since the total anion, except as noted below, Ca concentration, and buffer concentration were all constant, solution composition will be hereafter described by the cation concentration in milliequivalents per liter with the concentration units omitted. The ratio of final to initial cation concentration will be designated by a fraction. A change from 20 Na to 5 Na would be 1/4.

Bullfrogs (*R. catesbeiana*) and toads (*B. marinus*) were obtained from Lemburger, Oshkosh, Wisconsin. The frogs were stored at 4°C until used; the toads were kept fasting on wet sand at room temperature prior to use. Experiments were run at room temperature, 20–24°C. The animals were killed by decapitation and pithing. The

TABLE I
COMPOSITION OF INITIAL CONTROL SOLUTIONS

Mucosal		Serosal	
meq/liter		*meq/liter*	
20	Na	115	Na
0	K	5	K
2.2	Ca	2.2	Ca
20	SO_4	120	SO_4
2.2	gluconate	2.2	gluconate

Both solutions were buffered to pH 8.2 with 5 mM tris titrated to pH 8.2 with H_2SO_4.

abdomen was opened and the bladder, if empty, was partly filled with Ringer's through the cloaca. Each bladder half was freed from its peritoneal attachment and removed. The bladder halves were soaked in Ringer's until mounted. Unfortunately the bladders were generally too small to be divided into more than halves. This prevented the use of more desirable statistical designs for obtaining comparisons among ions. Also the error between bladder halves and the error among animals were confounded in the present experiments.

Two types of experiments were done. In one, henceforth called "serial dilution," the composition of the bathing solution was changed by addition of an equal volume of solution containing none of the ion whose concentration was to be altered. For example, when the concentration of the mucosal solution was 10 Na, 10 K, addition of an equal volume, usually 5 ml, of 10 K solution reduced the Na concentration to 5, but kept the K concentration constant. After allowing 3 to 5 minutes for mixing, one half of the resulting solution was removed. To prevent hydrostatic pressure differences across the bladder 5 ml of the control solution was simultaneously added to or removed from the opposite bathing solution. Several successive dilutions were made. In these experiments, the sulfate concentration changed.

A second type of experiment, called "total replacement," was done in which only the cation concentration of the bathing solution was changed by total replacement of the bathing solution. The chamber was rinsed twice with 5 ml of new solution in order to be relatively sure that the new solution would not be contaminated by

the previous solution. A typical total replacement experiment on the mucosal border of toad bladder is shown in Fig. 1.

During preliminary experiments, it was noted that when the serosal border was bathed with 120 K Ringer's and then returned to Na_2SO_4 Ringer's, the potential was then substantially higher than it was initially. For this reason, all toad bladders were exposed to K Ringer's for at least 15 minutes (Figs. 1 and 6) before the first control period. The bullfrog bladders were not treated with K-Ringer's. The mechanism of this increase in transbladder potential is unclear. When a steady potential was reached after pretreatment of the serosal surface with high K, the mucosal solution was replaced with an experimental solution in which part ot the Na was replaced

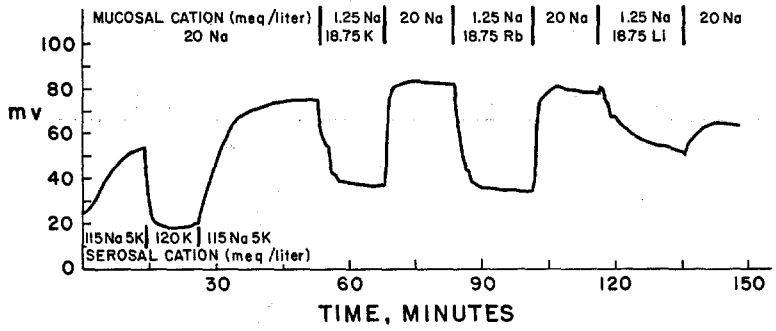

FIGURE 1. Effect of replacement of mucosal Na on toad bladder potential, redrawn from the original strip chart recording. Note the difference in potential upon return to the control solution after the serosa was bathed with 120 K. (Also see test and Fig. 6.) The artifacts associated with changing solution are clearly shown when the mucosal solution was changed from 20 Na to 1.25 Na, 18.75 K. In some experiments no artifacts were present (Fig. 6). The recovery following Li was poor.

by another alkali metal. When a new steady potential was reached, the mucosal solution was replaced with dilute Na_2SO_4 Ringer's. The potential was considered steady when it changed less than 1 mv per 5 minutes. Two additional experiments were done in the same manner with different alkali metals, each preceded and followed by a control period.

The criteria for acceptance of any experiment were an initial potential of 50 mv or greater and proper execution of the protocol. The distribution of initial potentials in carefully handled bladders from healthy animals appeared to show 90 to 95 per cent of all bladder potentials to be above 50 mv. The results are presented in terms of the change in potential, ΔV, which is the steady potential in the experimental period minus the potential during the preceding control period.

The meaning of the potential change, ΔV, and the validity of multiple replacement experiments on one piece of tissue have been discussed previously (2, 4).

RESULTS

A. *Na-K Sensitivity of Bullfrog Bladder—November*

Both serial dilution and total replacement experiments were done on bullfrog bladder. The mean initial control potential for all twenty six bladders used in

these experiments was 78.5 mv, SEM ± 3.00 mv. Final recovery periods were not carried out in all serial dilution experiments. In four experiments the Na sensitivity of the mucosal surface was tested by serial dilution. From the initial control condition (20 Na bathing the mucosa, 115 Na, 5 K bathing the serosa) the mucosal solution was replaced with 10 Na, 10 K. When a steady potential was reached, serial dilution of the mucosal solution was performed with 10 K to obtain successive dilutions of Na. Between each dilution sufficient time to achieve a new steady state was allowed. After the mucosal Na effects

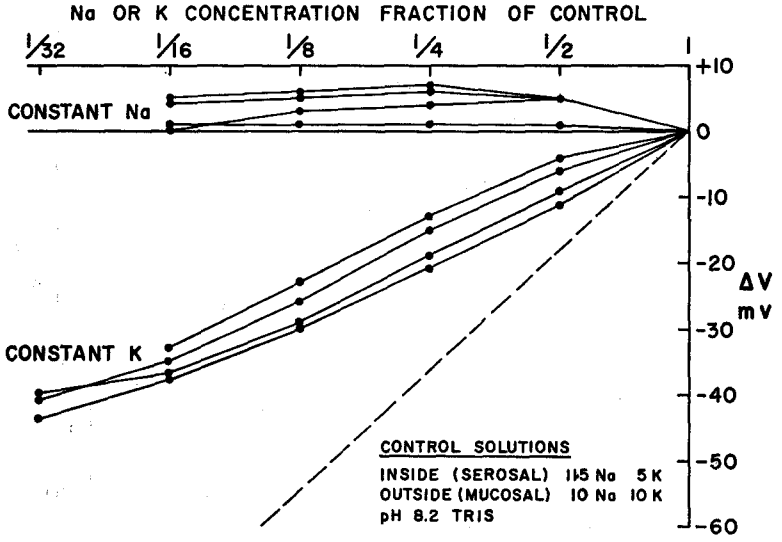

FIGURE 2. Effect on bullfrog bladder potential of dilution of mucosal Na with constant K and of K with constant Na. The ordinate is potential change in millivolts; the abscissa is the concentration expressed as a fraction of the original Na or K plotted on a logarithmic scale. The broken line has a slope of 59 mv per ten-fold change. The mucosal solution prior to the start of the serial dilution was 10 Na, 10 K. The serosal solution was 115 Na, 5 K throughout the experiment.

on the potential at constant K had been tested, the bladders were returned to 10 Na, 10 K and serial dilution with 10 Na was done. The combined results are illustrated in Fig. 2. The potential decreased as mucosal Na concentration decreased. By interpolation, the change in potential for a tenfold change in Na concentration should be approximately 30 mv. Dilution of K at constant mucosal Na concentration resulted in minor changes in potential unrelated to K concentration.

A similar experiment to determine the sensitivity of the serosal border was done on four bladders. The serosal solution was changed from the initial control solution (115 Na, 5 K) to 60 Na, 60 K. Successive twofold dilutions of K with constant Na resulted in increased transbladder potential; the change in potential per tenfold change in serosal K concentration was approximately

49 mv. Change in serosal Na concentration at constant K had little effect (Fig. 3). Two experiments on dilution of serosal Na at constant K were carried to only 1/4 and had to be terminated because the bladder potential began to decrease and did not recover.

The small changes in potential (1 to 7 mv) when mucosal K was decreased at constant Na (Fig. 2), and the small changes (−4 to +9 mv) when serosal Na was decreased at constant K (Fig. 3), may have been related to changes in anion concentration, ionic strength, or osmolality.

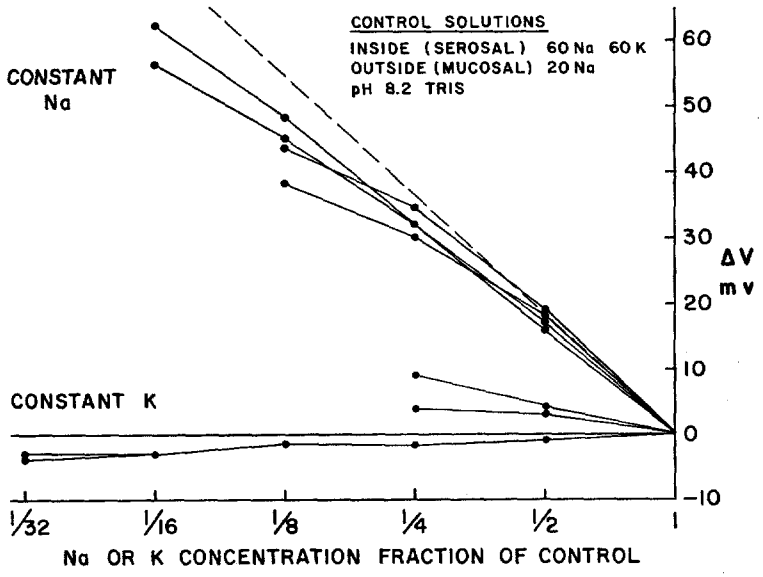

FIGURE 3. Effect on bullfrog bladder potential of dilution of serosal K with constant Na and of Na with constant K. The abscissa is the concentration, expressed as a fraction of the original Na or K on a logarithmic scale. The ordinate is the potential change in millivolts. The broken line has a slope of 59 mv per tenfold change. The serosal solution prior to starting the serial dilution was 60 Na, 60 K. The mucosal solution was 20 Na throughout the experiment.

To rule out the effect of changes other than those in the cation concentration, total replacement experiments (described in Methods) were done. The results of total replacement experiments on the mucosal border of eight bladder halves (mean initial control potential 78.1, SEM, ±5.24 mv; mean final control potential 72.5 ± 7.34 mv) are shown in Fig. 4. The results are similar to those of the serial dilution experiments. The data were reasonably well fitted by the constant field equation by the least squares procedure described by Lindley and Hoshiko (2). The constant field parameter, α, was calculated for the mean ΔV for a number of different bladders and is not the mean α from a number of individual bladders. In this experiment, α, which

may be interpreted to be the ratio of permeability coefficients P_K/P_{Na} or α_{KNa} for the mucosal border, was estimated to be 0.137. This is comparable to an α_{KNa} of the outside or epithelial border of 0.048 for bullfrog skin and 0.074 for leopard frog skin obtained by Lindley and Hoshiko (2). Thus, the mucosal border of bullfrog bladder appears to be relatively less Na-selective than the corresponding epithelial surface of frog skin.

The K sensitivity of the serosal border was tested in total replacement experiments on ten bladder halves (mean initial control potential 75.6 ± 6.28 mv; mean final control potential 77.0 ± 5.34 mv). The potential increase was

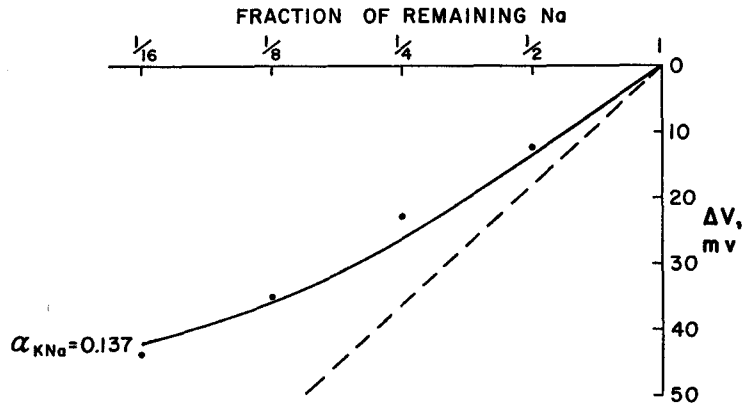

FIGURE 4. Effect of replacement of mucosal Na with K on bullfrog bladder potential. Each point is the mean of eight observations. The line is the least squares fit to the constant field equation. Mucosal control solution was 20 Na Ringer's. Serosal solution was 115 Na, 5 K Ringer's throughout. The standard errors of the means were all less than 3.5 mv. The 59 mv slope is shown by the broken line.

related to the change in K concentration (Fig. 5). The relative permeability ratio, α_{NaK}, was 0.064 compared to 0.097 and 0.09 for bullfrog and leopard frog skins. Thus, the serosal border of bullfrog bladder is also K-sensitive to about the same degree as the skin.

B. *Alkali Metal Selectivity of Toad Bladder—February and April*

Replacement experiments similar to those done in frog skin and bladder were repeated on the toad bladder to determine whether it was Na-sensitive at the mucosal border and K-sensitive at the serosal border. Figs. 1 and 6 show typical experiments on the mucosal and serosal borders of toad bladder. Li, K, or Rb was used to replace K in the experiments at the serosal border. In Fig. 1 the potential had reached 53 mv prior to treatment with 120 K inside. Upon return to the control Na solution, the potential increased to 75 mv. In Fig. 6 treatment with 120 K on the serosal surface increased the control

potential by 35 mv. Each bladder was tested with all three replacement cations at one of four Na or K concentrations: 1/2, 1/4, 1/8, 1/16.

Fig. 7 illustrates the results of replacement experiments (February) on the mucosal surface of twenty-four bladder halves. Each point represents the mean of six values. The mean initial control potential was 65.4 ± 2.35 mv; mean recovery potential was 51.4 ± 2.68 mv. K and Rb were almost equally poor replacements for Na. Because in frog skin Li in the outside bathing solution appeared to permanently alter the response of the epithelial border

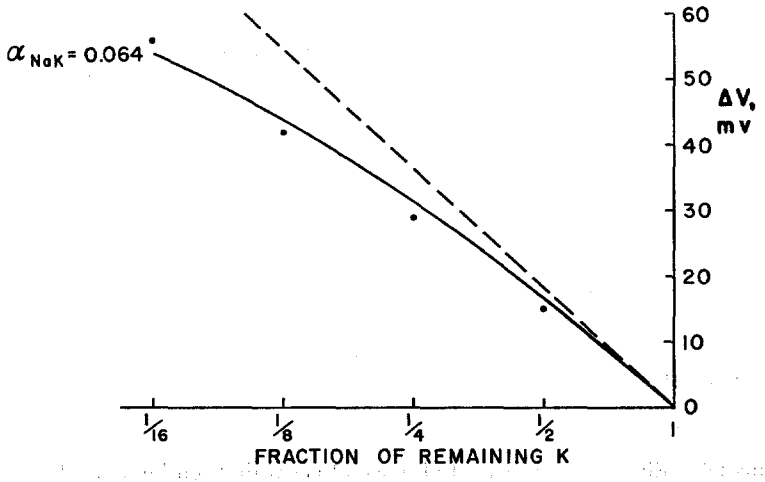

FIGURE 5. Effect of replacement of serosal K with Na on bullfrog bladder potential. Each point is the mean of ten observations. The line is the least squares fit to the constant field equation. Mucosal control solution was 20 Na Ringer's. Serosal control solution was 120 K Ringer's. The standard errors of the means were less than 2 mv except 1/8 SEM is 3.12. The 59 mv slope for a K diffusion potential is shown by the broken line.

(2, 7), Li treatment was done after K and Rb treatments (see Fig. 1). In the toad bladder, mucosal Li also caused a permanent change in the potential. The control potentials had declined slightly below the initial control values during the preceding treatments and just prior to exposure of the mucosa to Li were 44 to 91 mv, mean 61.5 mv. This is in contrast to the final control value reported above. Mucosal solution replacement with Li in toad bladder differs qualitatively from Li in frog skin. The sharp drop in potential when half the Na was replaced with Li contrasts with the results obtained in frog skin, but the 1/4, 1/8, and 1/16 points have a slope similar to that of frog skin. Relative permeability ratios calculated from the potential measurements were $\alpha_{KNa} = 0.204$, $\alpha_{RbNa} = 0.222$. A similar calculation for Li is probably not meaningful.

Because of the qualitative difference between the response to Li and the other alkali metals in toad bladder another experiment (April) was done in

which Li replacement for Na was tested. Only one experiment per bladder half was done. When Li replaced Na the potential fell rapidly for 10 minutes. At 30 minutes the change in potential was still greater than 1 mv in 5 minutes (a rate of change assumed to be consistent with a steady state). Recovery

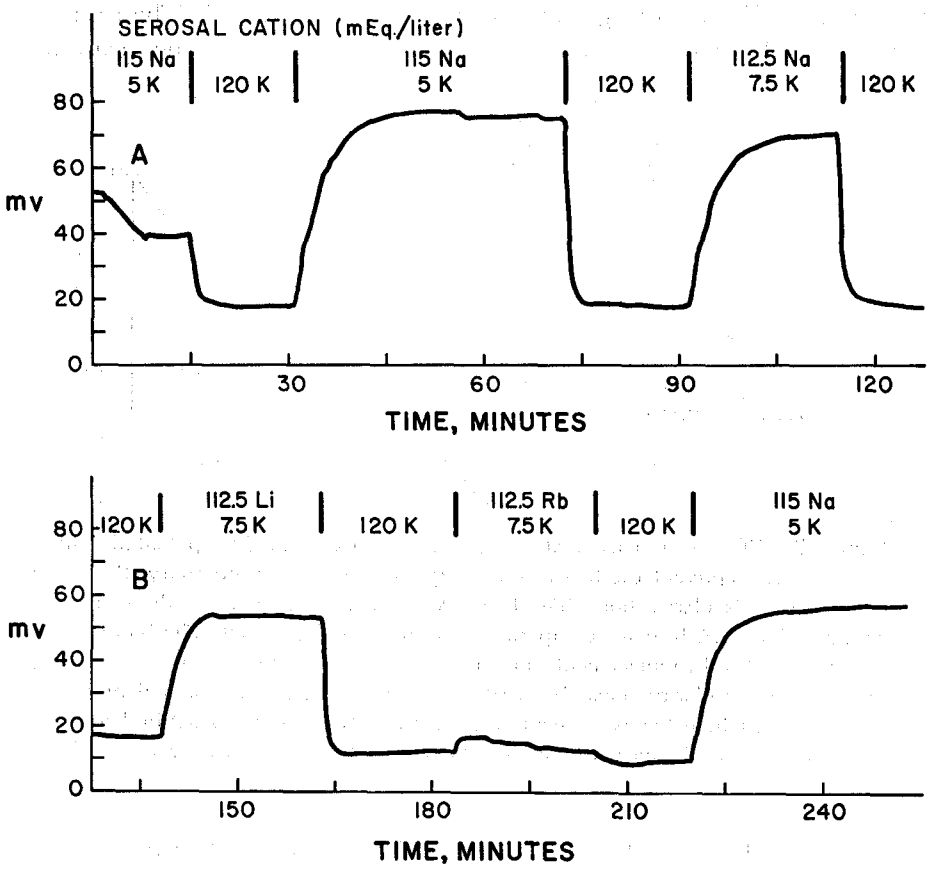

FIGURE 6. Effect of replacement of serosal K on toad bladder potential, redrawn from the original strip chart recording. The graph is split at 125 minutes. No artifacts due to changing solutions can be seen. When the serosal border was bathed with 120 K and then returned to the control solution, the potential was higher than in the original control solution.

upon return to the control solution was generally poor. (Mean initial control potential for the forty-eight bladder halves was 75.8 ± 2.19 mv; mean recovery potential 64.4 ± 2.48 mv.) The results are shown in Fig. 7 as open circles. The potential change at 1/2 the original Na exceeded that predicted by the Nernst equation. The potential change from all Na to Na + Li (control to experimental) could not be related to the change from Na + Li to Na

(experimental to control). Therefore, quantitative interpretation of these data appeared questionable and the constant field parameters are not reported.

In experiments at the serosal border, Li did not appear to injure the bladder. Therefore, the Li, Rb, and Na solutions were assigned in random order. At the serosal border of twenty bladder halves, both Li and Na were poor replacements for K. As in frog skin, Rb substituted well for K. The results are shown in Fig. 8. Each point is the mean of five experiments. The mean initial

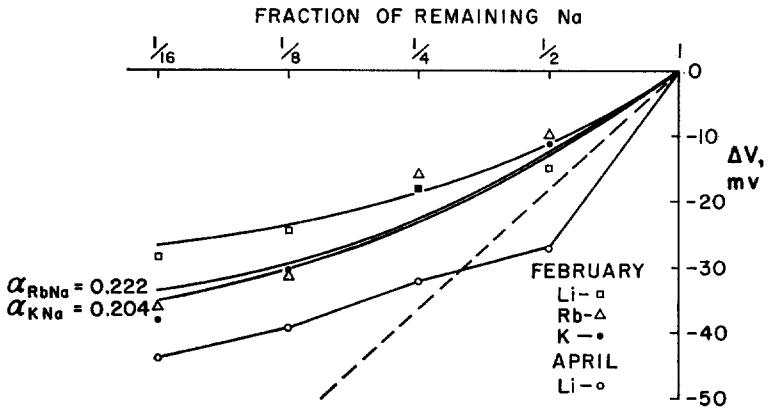

FIGURE 7. Effect of replacement of mucosal Na on toad bladder potential. The three upper curves represent the first series of experiments done in February. Each point is the mean of six observations. The lines are the least squares fit to the constant field equation. The 1/4 K point is superimposed on the 1/4 Li point. The bottom curve is the second set of Li replacements done in April. Each point (open circles) shown is the mean of twelve observations. Mucosal control solution, 20 Na. Serosal solution, 115 Na, 5 K throughout the experiment. The 59 mv slope is shown by the broken line. The standard errors of the means were all less than 4 mv, except February 1/4 Li, 5.94 and Rb 1/8, 4.65.

control potential was 66.9 ± 4.36 mv (one bladder did not recover from the 120 K, final potential 11 mv). The results are consistent with the hypothesis that the serosal surface of toad bladder is K-sensitive. Permeability ratios calculated from the potential change were $\alpha_{LiK} = 0.301$, $\alpha_{NaK} = 0.172$, and $\alpha_{RbK} = 0.956$.

Although the experiments were not designed to investigate the potential transients which occurred when the ionic composition of the bathing solutions was changed, an attempt was made to estimate the diffusion delay in toad bladder serosa from the half-time of the potential transient. The method used was that described by Lindley (4). The transient is assumed to result from the presence of an additional compartment between the bathing solution and the epithelial border in question. The potential at any time is related to the ion concentration at the cell border. From the constant field equation, a linear

equation relating the logarithm of the potential to the time can be obtained. $\mathrm{Ln}(y - y_\infty) = \ln a - \lambda t$, where $y = FV/RT$ at time, t, $y_\infty = FV_\infty/RT$ at the new steady state, a is a constant incorporating initial and final concentrations and the potential across the opposite border; λ is the reciprocal of the time constant; R, T, and F have their usual meanings. Calculation of the diffusion delay (8) caused by an unstirred layer of 1.2×10^{-2} cm, the thickness of the screens estimated with a micrometer, yielded a value of 6 seconds for the fundamental time constant. A 2 × increase in thickness (or a 5 × decrease in area available for diffusion) would only give about a 30 second time con-

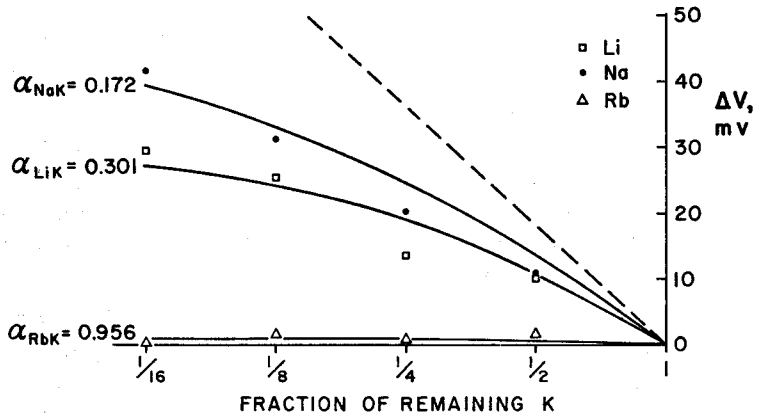

FIGURE 8. Effect of replacement of serosal K on toad bladder potential. Each point is the mean of five observations. The lines are the least squares fit to the constant field equation. Serosal control solution was 120 K and the mucosal solution was 20 Na throughout the experiment. The broken line is the 59 mv slope. The standard errors of the means were less than 3 mv except for 1/4 Li SEM is 3.50 and for 1/16 Na SEM is 3.50.

stant. In those experiments in which artifacts from changing solutions did not appear, the half-time could be estimated. Transients produced by potential decreases resulting from decreasing the mucosal Na concentration of four bladders averaged $t_{1/2} = 0.59$ minute. For the serosal border, transients produced by potential decreases due to increasing the K concentration of six bladders averaged $t_{1/2} = 1.11$ minutes; nine transients produced by potential increases due to decreasing the K concentration averaged $t_{1/2} = 2.75$ minutes. Thus, the half-times may be longer for the serosal surface than for the mucosal surface.

Resistance values were measured by passing a small constant current of 4 to 5 μ amp through the bladder in each direction and recording the potential change produced. In general, the resistances increased as the mucosal Na was decreased or as the serosal K was decreased. However, the changes were too small to allow quantitative interpretation. The resistances in the initial control solutions for bullfrog bladder ranged from 1000 to 2650 ohms cm^2; for

toad bladder, 2000 to 4000 ohms cm^2. These values are approximately the same as those estimated by Bentley (9) for frog bladder (*R. esculenta*) in Cl Ringer's, but somewhat higher than values calculated from the data of Frazier and Leaf (5), or from other experiments (Leb, unpublished) for toad bladder (*B. marinus*) in Cl Ringer's.

DISCUSSION

The amphibian urinary bladder proved to be a much more fragile preparation than the frog skin and several precautions were required. Extreme care was necessary to prevent mechanical trauma. Excessive stretching when mounting the preparation on the screens, touching the bladder, or imposition of a hydrostatic pressure difference, all caused permanent decreases in potential. Even with precautions to avoid injury when changing solutions, transient decreases in potential were observed frequently. The presence of Ca in the serosal solution was necessary to maintain the potential. Hays (10) previously has shown that the absence of calcium in the bathing solution caused the epithelial cells to become detached from the lamina propria. In contrast, the isolated frog skin survives well in Ca-free solution.

Bentley (9) has shown that the frog urinary bladder (*R. esculenta*) has a significantly higher potential and resistance when contracted, and that water movement in response to oxytocin is reduced. High K concentrations depolarize smooth muscle and cause contraction of the muscle. This is one possible explanation of the higher potential observed after treatment of the bladder with high K serosal solutions. Another possible explanation for the increase in bladder potential following exposure of the serosa to high K concentration, is that the intracellular electrolyte concentration is altered, thereby increasing the potential.

A dilute mucosal solution was used in the present experiments, because the bladder potentials appeared to be higher than those obtained with identical full (or half-) strength chloride or sulfate Ringer's bathing both mucosal and serosal surfaces. Studies on the frog skin indicated that the response of the potential to a change in solution composition at one border is affected by the composition of the solution bathing the opposite border. This interaction is reduced when a dilute solution bathes the epithelium and a nearly isotonic solution bathes the corium (11). In addition, the frog skin potential data obtained with dilute outside solution could be fitted well by the constant field equation (2). Although the conditions seemed the best of those tried, small systematic deviations occurred in fitting the constant field equation to the bladder data (see Figs. 5, 6, 8, 9).

The potential transients, while interesting, are too few in number and too selected to be considered representative data. Artifacts, possibly due to mechanical trauma, may have contributed to the calculated half-times. In frog skin experiments and in the bladder experiments where no artifacts appeared

in the records, the equation derived from a diffusion model could be fitted to the data with little difficulty. Perhaps a diffusion barrier, different on the mucosal and serosal borders, is largely responsible for the potential transient. This does not, however, exhaust the possibilities for interpretation of the time course of the potential change. The effect of change in K concentration at the serosal border may well affect nerves and smooth muscle, which in some way could contribute to the time course of the potential change. The possibility of time-dependent changes in the epithelial cell itself must not be ignored. Change in the mechanism of generation of the potential could occur with or without change in the intracellular electrolyte concentration. Extracellular spaces, demonstrated in toad bladder with the electron microscope, could function as shunt pathways for ions as proposed for frog skin (12, 13). Such shunting could affect the time course of the potential change. The small number of satisfactory observations make any conclusion hazardous; additional experiments under more ideal conditions would be necessary.

In contrast to the report of Frazier and Leaf (5), the present experiments are consistent with a K diffusion potential across the inner (serosal) border of the urinary bladder of toad and bullfrog. The results are similar to one of the lines of evidence originally presented by Koefoed-Johnsen and Ussing (1) in support of their model for frog skin. Because the serosal border is K-sensitive, lack of K sensitivity cannot be invoked as evidence for a direct electrogenic pump. However, the present experiments do not rule out the possibility that such a metabolically linked charge transfer mechanism, rather than an ionic gradient, is responsible for the bladder potential.

The present experiments showed that the mucosal (physiological outside) border of isolated toad and bullfrog bladder is Na-selective and the serosal (physiological inside) border is K-selective, similar to isolated frog skin as found by Koefoed-Johnsen and Ussing. The qualitative selectivity orders in toad bladder for the stable alkali metals are similar to those found in bullfrog and leopard frog skin (2).

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