J. Membrane Biol. 8, 189–203 (1972)

The Kinetics and Distribution of Potassium in the Toad Bladder

Arthur L. Finn* and Hugh Nellans

Department of Physiology, Yale University School of Medicine, New Haven, Connecticut and Department of Medicine, University of North Carolina School of Medicine, Chapel Hill, North Carolina

Received 22 September 1971

Summary. Short-circuited toad bladders were loaded with K^{42} from the serosal medium in a chamber stirred by rotating impellers. The chambers were washed with nonradioactive Ringer's, and all effluent was collected from the two chambers separately for 30-sec intervals for 30 min and counted. Count rate data were fitted to sums of exponentials and analyzed by the methods of compartmental analysis. There are at least two potassium compartments, with half times of 2.42 and 18.48 min. These compartments contain 2.01 and 7.93 μEquiv $\times$ 100 mg dry weight^{-1}, respectively, amounting to 45% of total tissue K. Determinations of the rate of buildup of tracer in the tissue after immersing the bladder in K^{42} Ringer's confirmed the fact that only a portion of tissue K exchanges even after one hr; thus the rest must have a considerably slower exchange rate. Fluxes at the inside border are far greater than at the outside, as predicted from electrophysiological data. Of the two tissue compartments, only the smaller and faster one appears to be related to Na transport, since only this compartment shows changes after Na removal (unidirectional serosal K fluxes decrease by some 50%) or after the addition of vasopressin (serosal fluxes and pool size increase by over two-fold). The results also are consistent with the operation of a 1:1 Na–K exchange pump at the serosal border.

The isolated urinary bladder of the toad is known to carry out the net transepithelial transport of Na against an electrochemical potential gradient. In addition to the operation of such a Na pump, it seems clear that this, like other oriented multicellular membranes, is able to maintain a high intracellular concentration of K. The mechanism of the maintenance of this K gradient is not entirely clear, but Koefoed-Johnsen and Ussing (1958), suggested the presence of a Na-K exchange pump at the serosal border of the epithelial cell, with subsequent passive diffusion of K into the serosal solution. Although it has been difficult to measure the movement of K

* *Present address:* Department of Medicine, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514.

across the serosal border of the toad bladder epithelial cells accurately, or across the analogous border of other epithelial systems such as the frog skin, it has been inferred that 1:1 Na-K exchange at the inside border is unlikely. Recent experiments in rodent renal tubules (Malnic, Klose & Giebisch, 1966) and in isolated frog skin (Biber, Aceves, Mandel & Curran, 1971) support this concept. In addition, there is little or no direct information as to the size of the pool of K which participates in such a Na-K exchange mechanism in the toad bladder, although in frog skin there is fairly good evidence that virtually all of the K is contained in a single pool (Curran & Cereijido, 1965). Such does not seem to be the case in toad bladder (Essig & Leaf, 1962; Kallus & Vanatta, 1970).

Recent developments in this laboratory (Finn & Rockoff, 1971) have made it possible to determine the size of the Na transport pool, and the fluxes of Na across the mucosal and serosal cell membranes. Because of the development of this technique, it seemed appropriate to study the kinetics of K movement in a similar manner, in the hopes of determining the fluxes of K into and out of the K pool or pools in the tissue and, if possible, to correlate K movements with Na movements.

Materials and Methods

The toads used in these studies were *Bufo marinus*, from Colombia, South America. They were obtained commercially from the Pet Farm, Miami, Florida, and kept on moist San-i-cel (Paxton Processing Company, Paxton, Illinois) prior to use. The toads were doubly pithed, and the bladders removed and placed in Ringer's solution composed, in mM/liter, of NaCl 109, KCl 2.5 or 5.0 (*see below*), $CaCl_2$ 1.0, $NaHCO_3$ 2.4 and glucose 5.6. Solutions were gassed with room air and had a pH of 7.8. The bladders were mounted in a chamber which has been described previously (Finn & Rockoff, 1971) and were kept short-circuited throughout all experiments. No bladders were studied unless the spontaneous transepithelial potential difference was 20 mV or greater.

Briefly, the chamber is one in which the media are stirred by rapidly rotating impellers, with separate entrance and exit ports on each side. After the short-circuit current reaches a steady state, radioactive K (as K^{42} Cl obtained from Cambridge Nuclear Corp. or from the International Chemical and Nuclear Corp.) is introduced into the serosal chamber. The final concentration of K in the loading solution is either 2.5 or 5.0 mM (depending upon the specific activity of the tracer as received from the vendor), in order to maintain a tracer concentration of at least 0.1 mC/ml. The tracer is allowed to remain in the chamber for at least 60 min, during which time nonradioactive Ringer's is continuously pumped through the mucosal chamber.

At the conclusion of this loading period, nonradioactive Ringer's is pumped through both chambers at a rate of 38.2 ml/min per chamber (nominal pump rate) for 1 min. Previously, we have shown that this procedure removed virtually all of the loading solution from the chamber (Finn & Rockoff, 1971). After 1 min, the flow rate is abruptly reduced to 7.8 ml/min per chamber, and all effluent is collected into test tubes mounted in a fraction collector. Fluid is collected from each side for 30-sec periods

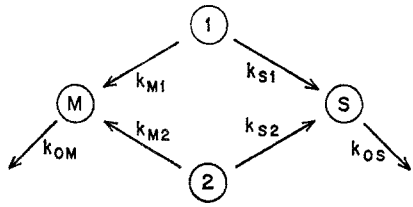

Fig. 1. Simple two-compartment tissue model. Mucosal (M) and serosal (S) chamber compartments are also indicated. The k's are rate coefficients for each of the unidirectional fluxes, shown as arrows; k_{0M} and k_{0S} refer to the rate coefficients for chamber washout on each side

for 30 min and subsequently the test tubes are placed in a well-type automatic gamma counter (Packard Instruments, Inc.) and counted. (Because of limitations imposed by the number of samples and the problems of decay, all samples from the first 30 collection periods, and alternate samples from the last 30 periods, are counted). Samples are counted long enough to give at least 10,000 counts, and all count rates are subsequently corrected for the decay involved in counting. At the completion of each set of experiments, the bladder is punched out of the assembly with a cork borer, dried to constant weight at 80 °C, and the dry weight obtained.

Theoretical Considerations

A compartmental model for the analysis of the data has been developed, as in the previous experiments regarding Na kinetics (Finn & Rockoff, 1971). As previously discussed, one can determine a lower bound on the number of compartments by fitting the data simultaneously with linear combinations of exponentials. In all of these studies, as shown below, a minimum of three exponentials was necessary, so that at least three compartments in the model were required. As shown before, one of these compartments is contributed by the chambers, which have a half time of emptying which depends upon the flow rate through the chambers. Each chamber has been shown to behave as a single well-mixed compartment: with a volume (mucosal or serosal) of 2.24 ml and a pump rate of 7.6 ml/min, the half time of washout of the chamber is approximately 12 sec.

Since the chamber accounts for one of the exponentials in the fit to the data, the minimum number of tissue compartments is two. As shown previously, the equations for such a system can be solved for each of the rate coefficients shown for the simple model in Fig. 1, and for the amount of radioactivity in the pools at time $t=0$. The computer program which we used (SAAM, developed by Berman, Shahn & Weiss, 1962) can also be used to simulate the model, and to study the number of possible configurations for a two-compartment tissue system. These connections include both series and parallel configurations and models including backflux from either or both chambers into the tissue compartment (Fig. 2). Extensive computations were carried out on four separate studies. In these studies, attempts were made to fit the data to models in which the two tissue compartments communicate. To do this, one of the additional rate coefficients (k_{21} or k_{12}) was allowed to vary, while the other was set at a fixed multiple such that the ratio k_{12}/k_{21} varied from 0.01 to 100. The reason for this is that the data cannot be fitted adequately when both of the extra parameters are allowed to vary without limit, since there then are too many independent variables to satisfy the data

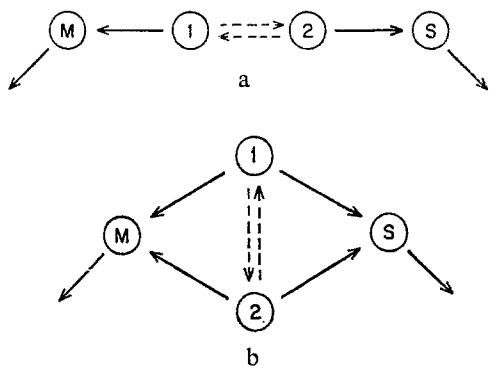

Fig. 2. Series (*a*) and parallel (*b*) models with communication between compartments. Backflux from mucosal or serosal chambers into tissue compartments is not shown, but could be added. Each possibility was tested with the computer, i.e., models with and without backflux from either or both chambers, and with and without uni- or bi-directional communication between tissue compartments (1 and 2)

(Berman *et al.*, 1962). It was found that the best fit occurred when there was no communication ($k_{12}=k_{21}=0$). The same was true when back flux from either or both compartments was permitted. Thus, as was the case with the Na kinetics previously described, a tissue system with two non-communicating compartments was the simplest model that would fit the data.

Results

Figs. 3 and 4 show the results of a typical washout experiment on a single bladder. The data are plotted semilogarithmically against time, and it is clear from inspection that at least two exponentials are required to describe the data. Computer fits to two (Fig. 3) and three (Fig. 4) exponentials are shown; it is clear that there are systematic deviations from the two-exponential fit, and that the fit is far better when three exponentials are utilized. Although the data could, of course, also be fit to four or more exponentials, the additional pair of coefficients and the additional exponential term could not be determined with precision in any experiment[1]. The fastest of the three components is due to chamber washout, as previously shown (Finn & Rockoff, 1971). The two remaining exponentials, namely those related to the washout of the tissue potassium, have exponential terms of 0.286 ± 0.019 and 0.0375 ± 0.0019 min^{-1}, or half times of 2.42 and 18.48

1 The computer program determines the values of the parameters and indicates the error associated with each determination. In each experiment fit to three exponentials, all coefficients and exponentials were determined with a standard error of 15% or less. For four-exponential fits, the extra coefficients and exponential were never determined with an accuracy of less than 150%.

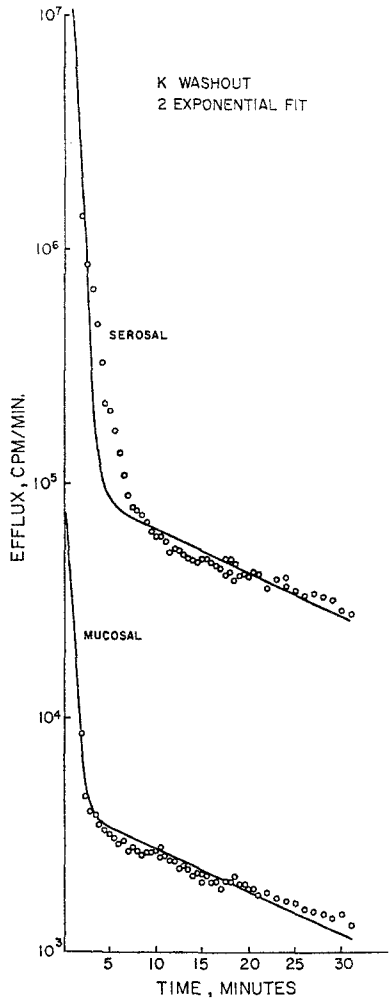

Fig. 3

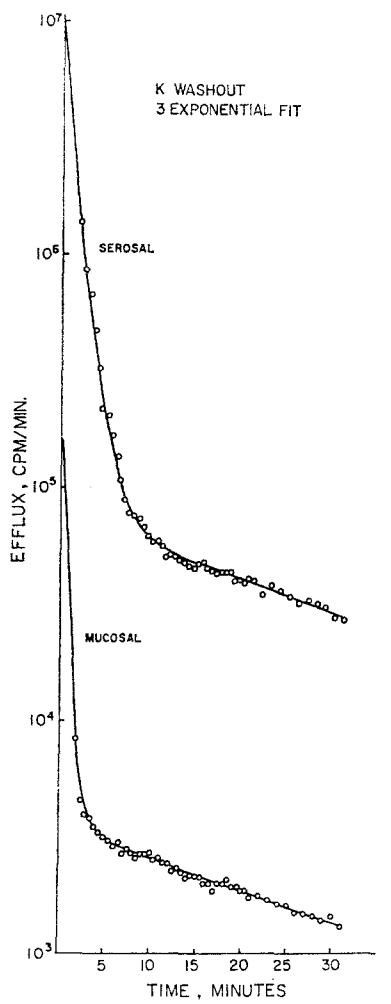

Fig. 4

Fig. 3. Two-exponential fit. Serosal (upper) and mucosal (lower) data (circles) are fit by the computer (solid line) to equations of the form: Serosal efflux $= A_1 e^{-\alpha_1 t} + B_1 e^{-\alpha_2 t}$; Mucosal efflux $= A_2 e^{-\alpha_1 t} + B_2 e^{-\alpha_2 t}$

Fig. 4. Three-exponential fit. The data points are from the same experiment as in Fig. 3. The equations are of the form: Serosal efflux $= A_1 e^{-\alpha_1 t} + B_1 e^{-\alpha_2 t} + C_1 e^{-\alpha_3 t}$; Mucosal efflux $= A_2 e^{-\alpha_1 t} + B_2 e^{-\alpha_2 t} + C_2 e^{-\alpha_3 t}$

min, respectively. From the computer output the values for the initial pools and rate coefficients are determined by using Eqs. (6) through (13) of Finn and Rockoff (1971). To determine the unidirectional fluxes and the K content of each compartment, we assume first, that a steady state for tracer

has been reached in each of the compartments at the start of the washout. Conventions and definitions are as follows:

J_{ij} = flux into compartment i from compartment j, μEquiv × min^{-1} × 100 mg dry wt^{-1};

A_i = total potassium in compartment i, μEquiv × 100 mg dry wt^{-1};

P_i = total radioactivity in compartment i, cpm;

p_i^* = specific activity in compartment i, cpm/μEquiv.

By reasoning similar to that previously described, it can be shown that

$$J_{1S}p_S^* = P_1(k_{S1} + k_{M1})$$
$$J_{2S}p_S^* = P_2(k_{S2} + k_{M2}).$$

Following achievement of the steady state, tracer washout begins. Assuming that $P_i(t)$ are continuous functions and, in particular, are continuous at time $t=0$, and that tracer flux from serosal compartment to tissue is negligible during washout, these equations may be solved for J_{1S} and J_{2S} at $t=0$ since p_S^* is known and $P_i(0)$ and the rate coefficients can be calculated from the washout data. We now make the additional following assumptions: (1) a steady state for total K exists, and (2) there is no net movement of K across the short-circuited toad bladder. These assumptions imply that the unidirectional fluxes at both the serosal and mucosal borders of each compartment are equal to one another ($J_{ij}=J_{ji}$). From the relationship that $J_{ij}=k_{ij}A_j$ we can now calculate A_1 and A_2 from the measured fluxes and rate coefficients. From these values, and the rate coefficients k_{M1} and k_{M2}, the fluxes at the mucosal border may be determined.

Calculated Results

Results of all control experiments to date are tabulated in Fig. 5. Conditions employed to test the model will be shown in greater detail below; however, since in all experiments a control K washout was performed in conjunction with an experimental manipulation, the summary of all control experiments is shown in the figure. For comparison, similar data have been compiled for the Na transport pool, as previously determined (Finn, 1971; Finn & Rockoff, 1971), and are shown in Fig. 6. It is immediately clear that the sum of the two K pools is about equal to the Na pool. This was a surprising result since it is known from previous data (Finn, 1968) that total K in the non-inulin space of toad bladders is considerably higher than that of Na, in common with many other transporting epithelia. Furthermore, the K in these two compartments represents only about 45% of the

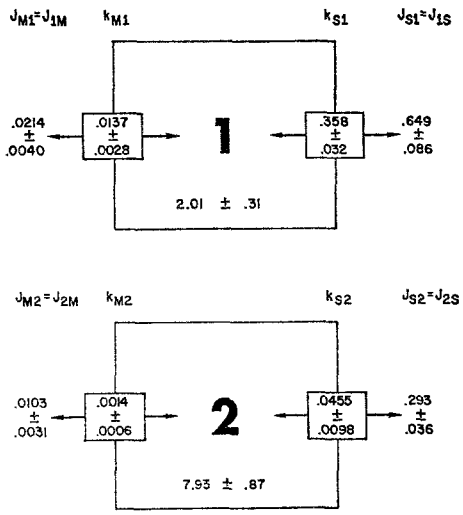

Fig. 5. Summary of control experiments. Fluxes (J_{ij}) are given in μEquiv × min ^{1} × 100 mg dry wt^{-1}, rate coefficients (k_{ij}) in min^{-1}, and pool contents (the numbers in the large boxes labelled 1 and 2) in μEquiv × 100 mg dry wt^{-1}. All values are given as means ± SEM

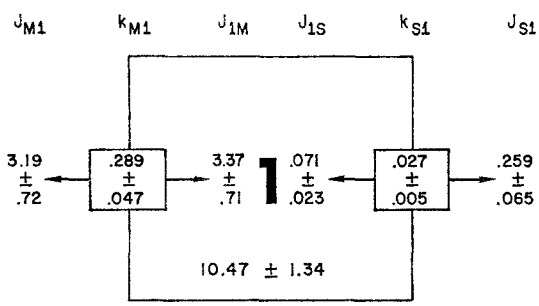

Fig. 6. Summary of data from Na experiments (Finn, 1971; Finn & Rockoff, 1971). In this case, the unidirectional fluxes at each border are not equal, since there is considerable net transepithelial Na movement

total K directly measured in whole tissue by flame photometry (Finn, 1968; Essig & Leaf, 1962). Although it has been shown in frog skin that virtually all of the K of the tissue is exchangeable with a single time constant (Curran & Cereijido, 1965), existing data for toad bladder suggest that in fact only a small portion of the total tissue K is exchangeable (Essig & Leaf, 1962; Kallus & Vanatta, 1970). Nonetheless, to be certain that this large fraction of tissue K which is not accounted for in these two compartments was not lost during the 1 min of rapid wash (when all effluent is discarded), the following experiments were performed:

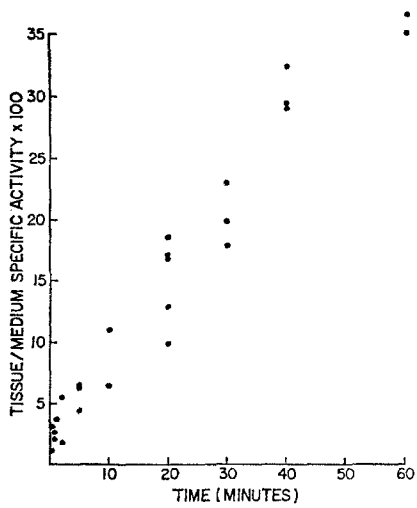

Fig. 7. Buildup of K^{42}. Each point represents a separate bladder sac whose serosal surface has been exposed to K^{42} Ringer's for the time shown on the abscissa

Bladder sacs were attached to glass cannulae, serosal side out, in beakers of Ringer's containing K^{42}, for periods of time from 5 sec to 60 min. The bladder was then removed, drained, blotted, placed in a test tube and counted. The bladder K was extracted in nitric acid and subsequently measured by flame photometry. Tissue/medium specific activity ratios were then calculated and plotted as a function of time, as shown in Fig. 7. It is clear that only a part of tissue K has exchanged even at 1 hr. Considering the difference in the technique employed, these findings are consistent with those described for the washout experiments. Thus, it seems evident from tracer build-up experiments that there is no rapid compartment of K which was lost in washout experiments during the first minute of rapid wash. Rather, one must conclude that the bulk of tissue K, which is not accounted for in the washout experiments, must be contained in a much slower compartment. Carrying the washout experiments for another 30 min did not allow distinction of a fourth component by the computer, nor did extending the loading period to 2 hr. Hence the bulk of tissue K is contained either in a non-exchangeable compartment or in one with extremely slow exchange rates.

As shown in Fig. 5, both K compartments display marked differences between serosal and mucosal permeabilities to K, with the fluxes and rate coefficient on the serosal side being some 30 times more than on the mucosal. These data are, of course, consistent with electrophysiological evidence that the serosal border of the toad bladder is relatively K permselective (Gatzy & Clarkson, 1965; Leb, Hoshiko & Lindley, 1965).

Of the two compartments, it seems likely that the smaller one is involved in the Na-K transport system since the half time of 2.42 min for the compartment is quite close to that for the Na transport pool (2.2 min). It seems quite unlikely that a compartment which turns over with a half time of 18.5 min would exchange with a much faster Na compartment.

To obtain further evidence as to which of the K compartments is involved in the postulated Na exchange mechanism, a series of experiments was performed to observe variations in kinetics under different conditions.

Since transepithelial Na transport requires the presence of Na in the mucosal medium, removal of Na should lead to a decrease in K movement at the serosal border if there is a Na-K linked pump. In each experiment to be described, then, a control washout was performed either before or after a washout was done in the absence of Na in the mucosal medium. In the latter experiments, choline (recrystallized from 95% ethanol) was substituted on a mole-for-mole basis for Na, and the Na-free medium was present for a minimum of 60 min before the washout was started. The results of these experiments are shown in Table 1. Na removal resulted in a decrease in the K fluxes at the serosal border of the fast compartment in every case. The size of the fast compartment, A_1, was not significantly altered (the difference shown was largely due to a single experiment, in which the control A_1 was 11.28; if this is excluded, the mean falls to 1.90 ± 0.24; in any case, inclusion of this experiment does not alter the statistical evaluation or the conclusions), nor were any of the other parameters involving this compartment. Furthermore, the removal of Na had no effect on the kinetics of the slow compartment. These data are compatible with the operation of a Na-K linked pump, but do not serve to establish any stoichiometric relationship between the two, since Na and K fluxes were not measured simultaneously.

To characterize the system further, the effect of vasopressin on the K fluxes was next determined. Since it is known that vasopressin stimulates Na transport by a mechanism which involves both an increase in entry across the mucosal side (Civan, Kedem & Leaf, 1966; Civan & Frazier, 1968; Finn, 1971) and a direct stimulation of the Na pump at the serosal border (Morel & Bastide, 1965; Finn, 1968, 1971), it was postulated that this hormone might be expected to increase K entry at the serosal border if there is in fact a Na-K linked pump. Accordingly, experiments were performed to test this. In half of the experiments the control washout was performed before the vasopressin study, and in half, the experiments were done in the opposite order. For the vasopressin experiments, K^{42} was added to the serosal chamber in the usual way, and vasopressin (Pitressin, Parke-

Table 1. Effect of mucosal sodium removal on potassium kinetics ($N=6$)

Medium	(a) Fast pool				
	J_{M1}	J_{S1}	A_1	k_{M1}	k_{S1}
Control	0.0234±0.0130	0.884±0.242	3.46±1.57	0.0171±0.0110	0.345±0.091
Na-free	0.0359±0.0298	0.425±0.083	1.99±0.40	0.0128±0.0089	0.252±0.050
Δ	−0.0126±0.0170	0.459±0.145	1.47±1.35	0.0043±0.0158	0.093±0.102
p	NS	<0.01	NS	NS	NS
	(b) Slow pool				
	J_{M2}	J_{S2}	A_2	k_{M2}	k_{S2}
Control	0.0097±0.0045	0.275±0.081	10.08±4.16	0.0048±0.0040	0.0311±0.0070
Na-free	0.0093±0.0045	0.314±0.113	8.70±2.19	0.0012±0.0005	0.0321±0.0064
Δ	0.0004±0.0010	−0.038±0.066	1.38±2.82	0.0036±0.0036	−0.0010±0.0094
p	NS	NS	NS	NS	NS

All results are expressed as means±SEM. J_{ij}= flux, μEquiv × min^{-1} × 100 mg dry wt^{-1}; k_{ij}=rate coefficient, min^{-1}; A_i=pool, μEquiv × 100 mg dry wt^{-1}; Δ=control, Na-free; NS=not significant.

Davis; final concentration 50 mU/ml) was added 30 to 45 min later. Washout was performed 30 to 60 min after this, at a time when the hormone-induced rise in short-circuit current was maximal. All experiments in this series were performed with a Na-free serosal medium, since under these conditions the response to vasopressin is greatly prolonged (Finn, 1971). Choline is used as a substitute for Na, and this change has no effect on K kinetics.

Results are given in Table 2. Again, it should be noted that changes are restricted to the faster of the two compartments. In each of the eight studies, there was an increase in serosal K fluxes and in the size of the pool. There was no significant change in the fluxes or rate constants at the mucosal side.

Discussion

The experiments described above indicate that there are two exchangeable K pools in the toad bladder; together, they account for a total of some 45% of total tissue K. The rest of the K is contained in a much slower pool which, because of its relative non-exchangeability, is almost certainly not involved in any Na-K exchange pump involved in transepithelial Na transport. Of the two pools described here, it seems quite likely that the faster of them represents that component of K which is involved in the Na-K transport system. One reason for this is that the half time for this compartment is quite close to that for the Na transport compartment previously described (Finn & Rockoff, 1971).

Although the data in Table 1 indicate strongly that there is a Na-K linkage, it is of interest to know the stoichiometry of this process. Essig and Leaf (1962) have suggested that the Na-K coupling ratio is considerably greater than unity in the toad bladder; however, their flux measurements were indirect, and there is no way to determine which K compartment was involved. Recently, Biber *et al.* (1971) have shown in frog skin that the ratio is about 3. However, as stated above, frog skin K behaves as though it is contained within a single compartment, so that its kinetics might not be expected to be similar to those in toad bladder.

The only entirely satisfactory way to measure the coupling ratio is to measure both Na and K kinetics simultaneously. Several attempts were made to do this, but the experiments were technically difficult. Another way of arriving at a coupling ratio would be to compare K influx or efflux at the serosal border with the net Na transport rate. On the average, net Na flux was found to be some 70% of the simultaneously measured unidirectional efflux at the serosal border, J_{s1} (Finn & Rockoff, 1971). For the present studies, we can plot net Na flux (from the short-circuit current)

Table 2. Effect of vasopressin on potassium kinetics ($N=8$)

Medium	SCC	(a) Fast pool				
		J_{M1}	J_{S1}	A_1	k_{M1}	k_{S1}
Control	0.604 ± 0.118	0.031 ± 0.009	0.348 ± 0.081	1.74 ± 0.43	0.0215 ± 0.0077	0.206 ± 0.023
Vasopressin	0.879 ± 0.149	0.110 ± 0.057	0.864 ± 0.259	4.05 ± 0.95	0.0220 ± 0.0064	0.201 ± 0.029
Δ	-0.275 ± 0.040	-0.079 ± 0.058	-0.516 ± 0.204	-2.31 ± 0.61	0.0005 ± 0.0102	0.006 ± 0.030
p	<0.01	NS	<0.05	<0.01	NS	NS
		(b) Slow pool				
		J_{M2}	J_{S2}	A_2	k_{M2}	k_{S2}
Control		0.0331 ± 0.0155	0.514 ± 0.086	12.20 ± 0.87	0.0026 ± 0.0010	0.0417 ± 0.0061
Vasopressin		0.0351 ± 0.0112	0.611 ± 0.093	16.52 ± 2.84	0.0020 ± 0.0004	0.0388 ± 0.0039
Δ		-0.0020 ± 0.0228	-0.097 ± 0.128	-4.32 ± 3.18	0.0007 ± 0.0013	0.0028 ± 0.0077
p		NS	NS	NS	NS	NS

Δ = control − vasopressin; SCC = short-circuit current, μEquiv $\times$ min^{-1} $\times$ 100 mg dry wt^{-1}; other abbreviations and conventions are as in Table 1.

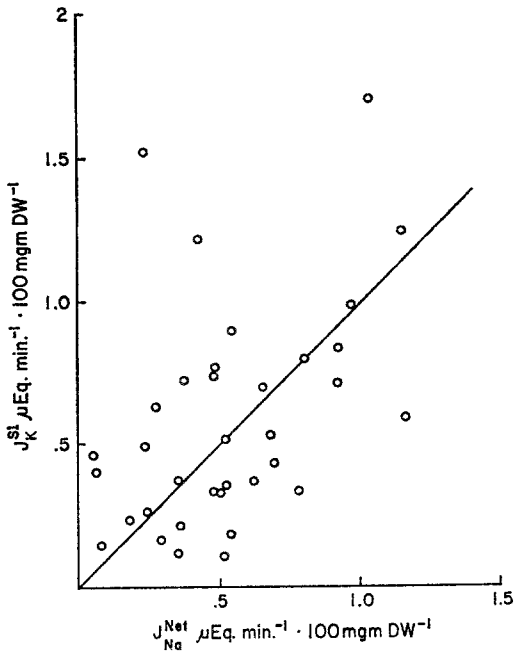

Fig. 8. Relationship between K and Na fluxes. The abscissa is the net transepithelial sodium flux calculated from the short-circuit current during the washout of K^{42}. The ordinate is the unidirectional K flux at the serosal border. Each point is from a separate control experiment. The line is that expected for equality of the two fluxes

against the K flux; such a plot is shown in Fig. 8. The solid line is the line expected for $J_{S1}^{K} = J_{net}^{Na}$, and the data, despite considerable scatter, suggest a Na:K ratio of near unity (even if one corrects the abscissa for the difference between SCC and unidirectional Na flux). The mean ratio J_{net}^{Na}/J_{S1}^{K} is equal to 1.02 ± 0.18. On the other hand, if one compares the unidirectional serosal efflux from the slow pool with the short-circuit current, the ratio is 2.55 ± 0.32.

Another way to evaluate the two-compartment tissue model is to compare the calculated transepithelial flux with that measured directly. The flux may be calculated from the unidirectional fluxes at the cell borders (Finn & Rockoff, 1971; Ussing & Zerahn, 1951) from the relation

$$J_{MS} = \frac{J_{M1} J_{1S}}{J_{M1} + J_{S1}}.$$

The flux may be measured directly by collecting timed samples from the mucosal chamber just prior to beginning the washout.

In eleven such experiments, the calculated serosa-to-mucosa flux, J_{MS}, was 0.0326 ± 0.0089 μEquiv × min^{-1} × 100 mg dry wt^{-1}, while the

measured flux was 0.0256 ± 0.0054. These values were not statistically different ($\Delta = 0.0070 \pm 0.0062$, $p > 0.5$). When the same calculations are made by utilizing the slow compartment fluxes, a value less than one-half of the measured fluxes is obtained (0.0108 ± 0.0030, $\Delta = -0.0149 \pm 0.0035$, $p < 0.01$). Although these data do not prove conclusively that compartment 1 is the transport K pool (since neither the role nor the pathway of transepithelial K movement is known), they do add some weight to the argument.

A further suggestion that the fast compartment is the compartment involved in Na-K exchange comes from the data in Tables 1 and 2, where the only changes seen are in that compartment. In the experiments in which Na transport was stopped by the removal of Na from the medium, there was a decrease in J_{S1}^{K} of 0.459 ± 0.145 μEquiv $\times$ min^{-1} $\times$ 100 mg dry wt^{-1}. From the data previously published (Finn & Rockoff, 1971), Na pump flux in control experiments was 0.259 ± 0.065. Considering that these values were obtained in different preparations, the possibility that at least some of the K flux may be through a pathway other than Na-K exchange, and assuming that the Na pump flux falls to zero when Na is removed from the medium, the values at least are of similar magnitude; they do not suggest a Na:K ratio that is greater than unity.

Furthermore, the increase in the unidirectional Na pump flux following ADH is of the same order as the increase in K fluxes shown in Table 2. The sodium data can be calculated from Finn (1971), and suggest an approximately fourfold increase in J_{S1}^{Na}, or an absolute increase of about 0.75 μEquiv $\times$ min^{-1} $\times$ 100 mg dry wt^{-1}, a value not far from the observed increase in J_{S1}^{K} of 0.50 shown here. Thus the data presented in this paper strongly suggest the following: (1) K fluxes at the inside and outside border of the toad bladder may be measured; (2) the inside border is far more permeable to K than is the outside, as indicated previously by electrophysiological data; (3) there are two K compartments which exchange with the serosal medium; these comprise only about 45% of total tissue K; (4) although there are two compartments, only one of them seems to be related to Na transport, since only this compartment is affected by removal of Na or by the addition of vasopressin; (5) available evidence is consistent with the operation of a 1:1 linked Na:K pump at the serosal border, though it is not yet possible to determine the stoichiometry of the linkage accurately.

Dr. Finn was the recipient of a Research Career Development Award from the National Heart Institute. This work was supported by USPHS grant no. AM-10025 and no. AM-15175.

References

Berman, M., Shahn, E., Weiss, M. F. 1962. The routine fitting of kinetic data to models: a mathematical formalism for digital computers. *Biophys. J.* **2**:275.

Biber, T. U. L., Aceves, J., Mandel, L. J., Curran, P. F. 1971. Uptake of potassium across the serosal surface of the isolated epithelium of the frog skin. *Biophys. Soc. Abstr.* p. 76A.

Civan, M. M., Frazier, H. S. 1968. The site of the stimulatory action of vasopressin on sodium transport in toad bladder. *J. Gen. Physiol.* **51**:589.

Civan, M. M., Kedem, O., Leaf, A. 1966. Effect of vasopressin on toad bladder under conditions of zero net sodium transport. *Amer. J. Physiol.* **211**:569.

Curran, P. F., Cereijido, M. 1965. K fluxes in frog skin. *J. Gen. Physiol.* **48**:1011.

Essig, A., Leaf, A. 1962. The role of potassium in active transport of sodium by the toad bladder. *J. Gen. Physiol.* **46**:505.

Finn, A. L. 1968. Separate effects of sodium and vasopressin on the sodium pump in toad bladder. *Amer. J. Physiol.* **215**:849.

Finn, A. L. 1971. The kinetics of sodium transport in the toad bladder. II. Dual effects of vasopressin. *J. Gen. Physiol.* **57**:349.

Finn, A. L., Rockoff, M. L. 1971. The kinetics of sodium transport in the toad bladder. I. Determination of the transport pool. *J. Gen. Physiol.* **57**:326.

Frazier, H. S. 1962. The electrical potential profile of the isolated toad bladder. *J. Gen. Physiol.* **45**:515.

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.

Kallus, F. T., Vanatta, J. C. 1970. Compartmentation and exchange of potassium in the toad bladder. *Biochim. Biophys. Acta* **211**:61.

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.

Malnic, G., Klose, R. M., Giebisch, G. 1966. Micropuncture study of distal tubular potassium and sodium transport in rat nephron. *Amer. J. Physiol.* **211**:529.

Morel, F., Bastide, F. 1965. Action de l'ocytocine sur la composante active du transport de sodium par la peau de grenouille. *Biochim. Biophys. Acta* **94**:609.

Ussing, H. H., Zerahn, K. 1951. Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. *Acta Physiol. Scand.* **23**:110.