

In vitro Demonstration of Potassium Excretion by the Toad Bladder¹ (35467)

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The reabsorption of water by the amphibian urinary bladder was shown by Steen in 1929 (1). In 1958, Leaf *et al.* (2) reported net sodium reabsorption by the isolated toad bladder. We have published *in vivo* evidence (3) that the bladder per se excretes potassium. This report presents data confirming potassium excretion by the isolated toad bladder. Simultaneous bidirectional fluxes were measured using ⁴⁰K and ⁴²K. Because this technique is not readily available to all laboratories, results of measurements of unidirectional flux in paired hemibladders are also reported.

Materials and Methods. The source, manner of handling, manner of subjecting toads (*Bufo marinus*) to a potassium load, and analytical methods have been previously reported (3). All toads were pithed prior to surgical procedures. Heparinized plasma was obtained from potassium-loaded toads by cardiac puncture. The radioisotope, ⁴²K, as ⁴²K₂CO₃ or ⁴²KCl was supplied by NSEC Division of International Chemical and Nuclear Corp., Pittsburgh, Pa., or by ISO/SERVE Division of Cambridge Nuclear Corp., Cambridge, Mass. Isotopically enriched KCl containing 1.90% ⁴⁰K was supplied by the Oak Ridge National Laboratory, Oak Ridge, Tenn.

Gamma counting was done on a Baird

Atomic detector attached to a Nuclear-Chicago scaler-timer or on a Tracerlab spectrometer attached to a well-type scintillation detector. Radioisotope counting was done to within a statistical accuracy of 1.0%. Standard corrections for background and decay were applied.

Analysis of ⁴⁰K was done on a 13 in., 60° sector field, second order double focusing mass spectrometer, built and operated by the Mobil Research and Development Corp., Field Research Laboratory, Dallas, Texas.

Simultaneous bidirectional flux experiments. Hemibladders dissected from potassium-loaded or nonloaded toads were mounted between plastic chambers of 2.0-ml volume each. The bladders were equilibrated for 30 min with unlabeled media. The chambers were then drained and rinsed twice with potassium-free Ringer solution. The chambers were filled simultaneously with the experimental solutions. The mucosal medium was a Ringer solution, made up with 1.90% enriched ⁴⁰K as the sole potassium source. The serosal medium was toad plasma with ⁴²KCl added to it. The total elemental potassium concentrations of the two solutions were equal. The experimental solutions were oxygenated prior to use.

In 7 measurements on 4 preparations, both the bladders and plasma used were from potassium-loaded toads. In a second group of 8 measurements on 4 preparations, the bladders and plasma were from nonloaded toads.

After the experimental exposure period, aliquots of both bathing media were taken for total elemental potassium concentration determinations. Gamma counting of ⁴²K was done on an aliquot of the mucosal bath. Mass

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spectrographic analysis of the percentage composition of ^{40}K was done on an aliquot of the serosal bath.

K Flux derived from ^{40}K flux measurements. To evaluate the accuracy of the method of determining increments above natural abundance of the percentage composition of ^{40}K , 3 control determinations were done on solutions made by adding 1.90% enriched ^{40}K to KCl solutions with an assumed natural abundance of 0.0118% (5). The average of these 3 determinations was 1.93% below the calculated values, with a maximum error of -2.99%.

The calculation of K flux based on the measured flux of ^{40}K was done in the following manner. The increment in percentage composition of ^{40}K ($\Delta\% ^{40}\text{K}_s$) in the serosal bathing medium was determined by mass spectroscopy; the percentage composition of ^{40}K ($\% ^{40}\text{K}_m$) was determined by mass spectroscopy, and the amount of elemental K in the serosal medium was determined by flame photometry (K_s μEq). The K flux was calculated as follows: $(\Delta\% ^{40}\text{K}_s \times \text{K}_s \mu\text{Eq}) \div (\% ^{40}\text{K}_m) = \mu\text{Eq of K}$; where $\mu\text{Eq of K}$ is the flux per bladder per duration of the flux period.

Unidirectional flux experiments. Three series of experiments were done by the technique of comparing unidirectional fluxes measured in opposite directions in paired hemibladders mounted between plastic chambers. Series 1 was done on bladders from potassium-loaded toads using plasma from potassium-loaded toads as the bath on both sides. Series 2 was done on bladders from nonloaded toads using plasma from nonloaded toads on both sides. Series 3 was on bladders from loaded-toads using Ringer solution on both sides of the bladder. All baths were oxygenated by shaking with 100% O_2 prior to use. The bath then has sufficient O_2 in solution so that 1 ml of bath will supply the bladder for over 2 hr, based on Leaf and co-workers' report (4) of the rate of O_2 consumption by the bladder.

In series 1, the bladders were equilibrated with unlabeled loaded plasma for 30 min prior to the flux period. The flux was determined for each of two 30-min periods in both

series 1 and 2. In series 3 the bladders were equilibrated with Ringer solution with ^{42}K label in the appropriate bath for 30 min. The flux was then determined for two 15-min periods. The chamber volume was 2.0 ml on each side in series 1 and 2, and 1.0 ml on each side in series 3.

Series 1 and 2 were carried out over many weeks and the magnitude of flux varied markedly from one bladder to the other. Series 3 was carried out over a 3-week period on toads obtained at one time. In addition, the potassium concentration of the media was 3.0 meq/liter in all experiments; whereas in Series 1 and 2, the concentration varied considerably.

Results. Simultaneous bidirectional flux. The results of the simultaneous determination of bidirectional fluxes of potassium across both loaded and nonloaded hemibladders are shown in Table I. A net serosal to mucosal

TABLE I. Simultaneously Determined Bidirectional Fluxes of Potassium.

All values in nanoEq/100 mg of bladder wet wt \times min.

Expt. no.	S \rightarrow M ^{42}K	M \rightarrow S ^{40}K	Δ (S \rightarrow M) - (M \rightarrow S)
K-Loaded toads			
1B	10.8	0.9	9.9
2A	35.7	14.1	21.6
B	100.5	28.7	71.8
3A	9.2	0.9	8.3
B	25.0	2.3	22.7
4A	51.1	16.3	34.8
B	120.0	46.0	74.0
Av			34.72 ^a
SEM			± 10.39
Non-K-loaded toads			
5A	3.809	1.402	2.408
B	7.848	0.703	7.145
6A	4.982	0.568	4.414
B	5.683	1.005	4.678
7A	2.241	0.470	1.771
B	2.825	0.280	2.545
8A	1.311	0.111	1.200
B	2.239	0.088	2.151
Av			3.2889 ^a
SEM			± 0.6990

^a (*t* test *p* < 0.01).

flux was observed in all experiments, and the average net flux in the potassium-loaded group is significantly greater than that of the nonloaded group (t test $p = 0.005$). Potassium concentrations of the bathing media were 6.22 mEq/liter in the nonloaded group and 8.93 mEq/liter in the loaded group.

Paired hemibladders. Series 1. There was a net serosal to mucosal flux in 10 of 13 sets of flux determinations. The serosal to mucosal (S \rightarrow M) flux was 80.3 ± 32.7^3 nanoEq/100 mg of bladder \times min and the mucosal to serosal (M \rightarrow S) flux was 62.4 ± 32.2 nanoEq/100 mg of bladder \times min. The mean difference was 20.5 ± 16.3 nanoEq/100 mg of bladder \times min (t test $p > 0.20$, binomial $p = 0.046$).

Series 2. On 9 of 11 sets of determinations on bladders from unloaded toads, the mean S \rightarrow M flux was 16.2 ± 6.1 nanoEq/100 mg \times min and the mean M \rightarrow S flux was 7.8 ± 2.5 nanoEq/100 mg \times min. The mean difference was 8.45 ± 6.02 (t test $p > 0.10$, binomial $p = 0.033$).

Series 3. In 39 pairs of measurements on 20 bladders the S \rightarrow M flux averaged 2.69 ± 0.43 and the M \rightarrow S flux averaged 2.42 ± 0.38 . The average difference was 1.27 ± 0.33 (t test $p < 0.005$).

Discussion. Both ^{40}K and ^{42}K are available for bidirectional flux measurements of potassium. ^{40}K emits both beta and gamma radiation but its half-life is 1.4×10^9 years, and it is classified as a stable isotope. In a flux experiment using 100% enriched ^{40}K , there would not be sufficient radioactivity moving across the bladder in a reasonable period of time for accurate determination of the flux. Because of this, the ^{40}K concentrations were determined by mass spectroscopy.

In the simultaneous bidirectional flux measurements, it would have been desirable to reverse the directions of the particular isotope fluxes in half of the experiments to evaluate the possibility of an isotope separation phenomenon occurring. However, the addition of the 1.90% enriched ^{40}K to plasma on the serosal side would either decrease the percentage enrichment to unacceptable levels, or raise the potassium concentrations to non-

physiological levels. Hence, ^{40}K was used in Ringer solution on the mucosal side and all ^{40}K fluxes were in the mucosal to serosal direction, and all ^{42}K fluxes were in the serosal to mucosal direction.

Wagener and Landmann (6) report that isotopic separation of ^{39}K and ^{41}K by the erythrocyte membrane transport system probably does not occur, but on the basis of their analysis they could only be sure that if it did occur, it was not greater than 1.1%. Thus, if one assumes that no greater separation of ^{40}K and ^{42}K occurs across the toad bladder than occurs with ^{39}K and ^{41}K across the red cell membrane, then it is obvious that the magnitude of flux asymmetry observed in our experiments was not due to isotope separation.

The accuracy of the calculated fluxes of ^{40}K and ^{42}K was evaluated. Using the 1.90% enriched ^{40}K , the increment in percentage composition of ^{40}K in the sample due to flux above natural abundance of ^{40}K in the plasma can be determined to within 2.0% accuracy. The combined errors of the ^{40}K flux determination is calculated to be 5.4%. Similarly, the calculated error of the ^{42}K flux is 3.5%. With these levels of error and the observation that the net flux exceeded the mucosal to serosal flux by 100% in all experiments, it is clear that the degree of flux asymmetry observed should not be due to experimental error.

In the simultaneous bidirectional flux, the greater net K flux in bladders from loaded toads than in bladders from nonloaded toads indicate that this K excretion is increased in response to the need for increased K excretion. This raises the question, "What is the mechanism by which this rate of excretion is controlled?"

The fact that the net difference in flux on bladders from loaded toads in which Ringer solution was on both sides of the bladder (series 3, unidirectional flux), was much lower than in either the series 1 bladders or in the bidirectional flux loaded bladders, both of which had plasma on them, suggests that there is a factor in plasma which stimulates the bladder to excrete K.

On the other hand, two series reported by

³ Av \pm SEM.

Frazier and Vanatta (7) in which only the S \rightarrow M flux was measured did not show any difference in spite of the fact that plasma was on the serosal side in one series and Ringer solution was on both sides in the other series. In these two series the S \rightarrow M flux with the plasma was 17.9 ± 6.0 , and the S \rightarrow M flux with the Ringer solution was 16.4 ± 2.6 nanoEq/100 mg \times min.

Having found that the toad excreted potassium *in vivo*, we chose to do these experiments in the nonshort-circuited state. Without such data, it would be impossible to interpret data from bladders in the short-circuited condition, especially if no significant potassium excretory flux were found.

We have determined the S \rightarrow M flux in chemically short-circuited bladders. These experiments are reported by Frazier and Vanatta (7). The potential difference across the bladder was reduced to near zero by placing a sodium-free Ringer solution on the serosal side of the bladder. This procedure did not alter the S \rightarrow M potassium flux. In addition, these authors measured the S \rightarrow M flux of potassium before and during stimulation of the bladder by placing vasopressin in the serosal bath. Although the characteristic increase in the electrical potential across the bladder due to stimulation of Na transport was observed, the S \rightarrow M flux of K did not increase. These observations indicate the K excretion by the bladder is not coupled to sodium reabsorption either directly or indirectly.

The unidirectional flux determinations in paired hemibladders are reported so the reader might know the limitation of this method. This method can give wide range of values,

as shown by the results of series 1. The variation was much less in series 3. This is attributed to the fact that the experiments were carried out over a shorter calendar period, and all the animals were from a single shipment.

Attempts to obtain paired unidirectional flux measurements in the same bladder at different times were unsatisfactory. The radioactive K could not be readily eluted from the hot side of the bladder after the first flux determination.

Summary. The *in vitro* studies reported confirm the previous *in vivo* report of potassium excretion by the toad bladder. Subjecting the toad to a potassium load augments the *in vitro* excretion of potassium, also confirming this *in vivo* observation. In addition, the determination of net potassium flux using simultaneously determined bidirectional fluxes of ^{40}K and ^{42}K across a single piece of bladder tissue is described.

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