

# Exchange of Phospholipid-Bound Na in the Toad Bladder with $^{24}\text{Na}^*$ (34129)

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Solomon and Vanatta (1) reported a correlation between the effects of seven enzymes on the rate of sodium transport in the proximal convoluted tubule of the rat kidney, and the effect of these same enzymes on the sodium binding by phospholipids extracted from the kidney. They interpreted this as evidence "that phospholipids play a critical role in salt transport by the proximal tubule." Vanatta (2) reported on studies of Na binding by phospholipids extracted from the toad bladder. If the bladder is incubated with a reduced [Na] in the mucosal bath, the Na in the phospholipids extracted from the bladder is reduced. The reduction correlates with the known effect of reduced [Na] in the mucosal medium on Na transport.

In this paper I report studies on the urinary bladder of *Bufo marinus* in which  $^{24}\text{Na}$  has been used to determine the relationship of the phospholipids to Na transport.  $^{42}\text{K}$  has been used to test binding of another cation.

If the phospholipids are transporting sodium, the sodium they contain would by definition be a part of the sodium transport pool. It is known that the sodium transport pool is about 11% (3) of the total sodium of the bladder, and that it labels readily from the mucosal side, and poorly if at all from the serosal side. It follows then that the specific activity of the sodium in the phospholipids would be higher than the average specific activity of the Na for the whole bladder, if this sodium is in the process of being transported.

If the labeling of the phospholipids is a random process occurring during the extrac-

tion process, then the phospholipid should be no more likely to obtain labeled sodium from the transport pool, than it would to obtain unlabeled sodium from the serosal tissue, and the specific activity of the sodium in the extract would be the same as the average specific activity of the sodium in the whole bladder.

*Materials and Method.* Urinary bladders of *Bufo marinus* were used throughout. These were excised, and half bladders cannulated and tested for leaks. When exposures were of the mucosal surface alone, the bladders were everted before cannulation. The cannulated bladder was filled with 6–10 cc of unlabeled Ringer solution. It was blotted free of excess solution on the outside, and then immersed in  $^{24}\text{Na}$ -labeled Ringer solution. The bladder was removed from the solution. The outside was again blotted free of excess solution and the inside emptied. It was then filled with air and frozen by immersing in liquid  $\text{N}_2$ . The exposure time was measured from the initial immersion in labeled solution until the time of freezing. The preparation for freezing took about 40 sec, so the actual time of immersion in labeled Ringer solution in the 1 min experiments was about 20 sec.

After freezing, the bladders were lyophilized, extracted, and the extract purified as previously described (2). This extract contained the phospholipids with the bound cations. The  $^{24}\text{Na}$  and sodium content or the  $^{42}\text{K}$  and potassium content of this extract was determined. The bladder was then immersed in water for at least 1 hr. The  $^{24}\text{Na}$  and sodium content or the  $^{42}\text{K}$  and potassium content of the water was determined.

The  $^{24}\text{Na}$  or the  $^{42}\text{K}$  content of both the water and of the phospholipids was deter-

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mined by using a well-type scintillation detector. Sufficient counts were collected to assure 1% accuracy of the counting procedure. The sodium or potassium content of the phospholipids in solution in 70% ethanol, and of the water extract was determined on a model B Beckman spectrophotometer with a flame attachment as previously described (2).

The specific activity of the lipid extract is defined as the ratio of  $^{24}\text{Na}$  concentration to the Na concentration in the purified lipid extract, which contains phospholipids with the bound sodium. The specific activity of the bladder is defined as the ratio of the  $^{24}\text{Na}$  concentration to the Na concentration in the water extract of the bladder. The "ratio of specific activities" of a bladder is defined as the specific activity of the lipid extract divided by the specific activity of the bladder.

In the studies with  $^{42}\text{K}$ , the specific activities and the ratio of specific activities are similarly defined with respect to the concentrations of  $^{42}\text{K}$  and K determined in the samples.

*Experimental Results. Control experiments.* In order to evaluate the accuracy of the determinations, bladders were exposed to identical  $^{24}\text{Na}$ -labeled Ringer solutions on both mucosal and serosal surfaces for a period of 20 min. This time is sufficient to expect near steady state conditions to exist with respect to  $^{24}\text{Na}$  movement in the serosal tissue on the one side of the bladder, and into the epithelial cells lining the mucosal side of the bladder. The sodium which then labels the phospholipids, regardless of how or when it attaches itself to the phospholipid molecules, will be expected to have the same specific activity as the bladder. In ten experiments, the ratio of specific activities observed ranged from 0.92 to 1.09 with an average of 0.975.

*Label from mucosal side.* Twelve everted half-bladders were exposed to  $^{24}\text{Na}$ -labeled Ringer solution on the mucosal side for 1 min. The ratio of specific activities varied from 0.74 to 1.31 and averaged 1.134. Eight of the values were higher than 1.09, the highest of the control series. The Mann-Whitney U test (4) comparing this series

with the control series was done ( $p < .01$ ).

*Label from serosal side.* Ten half-bladders were exposed to  $^{24}\text{Na}$ -labeled Ringer solution on the serosal side for 1 min. The ratio of specific activities varied from 0.98 to 1.09 with an average of 1.03. Mann-Whitney U tests comparing this series with the control series ( $p > .05$ ), and with the 1-min mucosal exposure ( $p < .05$ ) were done.

*Potassium-42 exposure.* Six half-bladders were exposed to  $^{42}\text{K}$ -labeled Ringer solution on the mucosal side for 1 min. The ratio of specific activities ranged from 0.36 to 1.11, averaging 0.58. The values were below 0.75 in five of the six experiments. Six half-bladders were similarly exposed on the serosal side for 1 min. The ratio of the specific activities of potassium ranged from 0.45 to 0.79 and averaged 0.52.

*Discussion.* The toad bladder, under conditions of exposure to Ringer solution on both sides contains 86.9  $\mu\text{eq/g}$  tissue water and the sodium transport pool is 9.3  $\mu\text{eq/g}$  of tissue water (3). The sodium transport pool is 10.7% of the sodium in the bladder.

The rate of labeling of this transport pool has a half-time of 1.0–4.6 min. Thus a small amount of radioactive sodium would be expected to have entered the bladder in 1 min of mucosal exposure. The phospholipid is known to be in the epithelial cell (2) and not in the serosal tissue.

It is probable that the binding of sodium by the phospholipids under the conditions of extraction is by incorporation of the Na into a micelle which has the lipophilic portions of the molecules oriented outward in the lipid solvents used. The cations would then be trapped in the center of the micelle and would be associated with the phosphate group.

The question must be asked as to whether the relationship of the Na to the phospholipid molecule existed in the living tissue, or came about during the extraction process. If the binding occurs in the solvent phases during the extraction process, there is no reason for the phospholipid to be more likely to pick up a sodium ion in the transport pool, than one in the serosal tissue. If such a random labeling occurred, then the specific activity

would be the same for the phospholipid-bound Na in the lipid extract as that of the tissue.

On the other hand, the phospholipid could be combined with Na which is in the process of Na transport at the instant the bladder is frozen. The binding could be in a micelle formed by the phospholipid which could be the carrier. On the other hand, the phospholipids could be lining the sides of a pore in the membrane, and the sodium could be being moved through this pore by whatever the forces are that produce active transport. In either case, the extraction procedure could then remove these phospholipids with a preponderance of the Na that is attached to them at the instant of freezing still attached to them at the end of the extraction and purification process.

The control experiments indicate that the combined errors of the four measurement,  $^{24}\text{Na}$  of phospholipids,  $^{24}\text{Na}$  of bladder, Na of phospholipids, and Na of bladder can give errors as great as 0.09 in the ratio of specific activities.

The mucosal labeled experiments indicate that the specific activity of sodium in the phospholipids of the extract is higher than the average specific activity of sodium in the whole bladder. The 1-minute time is short enough that the preponderance of the  $^{24}\text{Na}$  will be in the transport pool which is in the epithelial cell. The delays of appearance of  $^{24}\text{Na}$  on the serosal side published (3) would indicate that very little if any  $^{24}\text{Na}$  would be on the serosal side. The high specific activity of the extract then comes from the preferential labeling of the phospholipids from the transport pool.

On the other hand, in the 1-min serosal experiment, the transport pool should not be labeled to any extent, and yet there is  $^{24}\text{Na}$  in the extract. The published rates of movement of Na and K through the serosal extracellular fluid would indicate that the specific activity of sodium in this compartment would be nearly that of the serosal bath. If one accepts a carrier theory of active sodium transport such as published (5) and places the barrier or energy-requiring step on the mucosal side of the transport system, it is

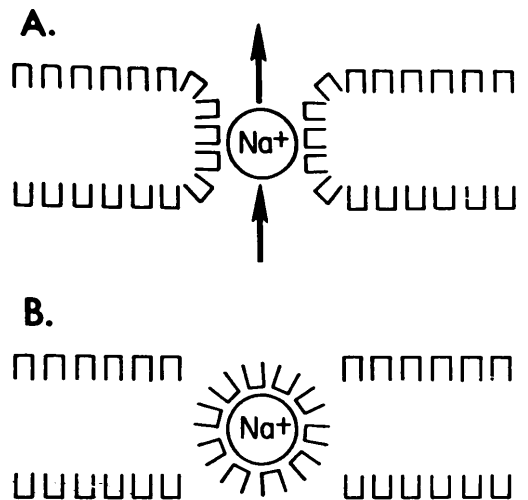


FIG. 1. A shows a pore in a membrane with a  $\text{Na}^+$  being transported through it. The walls of the pore are lined with phospholipids, the open end of the U being the lipophilic portion of the molecule, and the other end being hydrophilic, as classically diagrammed. B shows in concept how such a  $\text{Na}^+$  might be trapped in a micelle during the extraction procedure. The phospholipids are separated from supporting proteins, which are not shown. Those lining the pore now surround the sodium trapping it in the micelle. The other phospholipids would also be extracted, but are shown here in their original position.

then possible for  $^{24}\text{Na}$  to combine with the carrier molecule from the serosal side.

It is tempting to conclude that the phospholipids form a micelle containing Na which moves through the membrane in some manner and discharges the sodium on the serosal side. This would be in keeping with the recent work on the effects of valinomycin on K transport in sheep cells and other membranes. Tosteson (6) explained these effects on the basis of valinomycin admitting the K into a sheltered space in the center of a doughnut-shaped molecule, and then this complex moving through the membrane. In a micelle, the sodium would be even more sheltered in the hollow center of a sphere.

It must be remembered that if the Na were moving through a pore in the membrane it would be possible for the solvent to form a micelle of phospholipids around the sodium. It is probable that the phospholipids do not

exist in the bladder as such but as lipoprotein. This is suggested by the fact that it is necessary to have ethanol present to effect the extraction, presumably the protein-lipid bond of the lipoprotein is broken by ethanolysis. The manner in which Na in a pore would then be trapped in a micelle is shown in Fig. 1.

It is indeed possible that the phospholipids exist in the cell in the proximity of the transport pool, and the Na is trapped by them in this position, and that they have nothing to do with Na transport through the membrane. If this were the case, one would then expect a similar trapping of the radioactive K. The fact that bound sodium in the phospholipids labels preferentially from the transport pool suggests that these phospholipids play a role in Na transport. However, this binding can be explained equally well on a basis of a carrier model of the transport system, or on a pore theory of the transport system.

*Summary.* The phospholipids of the toad

bladder bind sodium. Using  $^{24}\text{Na}$  it is evident that the bound sodium can be more readily labeled with  $^{24}\text{Na}$  from the mucosal side of the bladder, than with  $^{24}\text{Na}$  from the serosal side. This observation supports the theory that the phospholipids play an important role in active sodium transport.

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