Compartmentation of the Sodium Transport Pool of the Toad Bladder[1] (34479)

JOHN C. VANATTA AND LEEARL ANN BRYANT

Department of Physiology, University of Texas, Southwestern Medical School, Dallas, Texas 75235, and Collins Radio Co., and Southern Methodist University; Institute of Technology, Dallas, Texas 75222

The toad bladder actively transports sodium from the mucosal to the serosal side. This transport is a function of the epithelial cells. The prevailing working hypothesis is that sodium is passively transported through the mucosal plasma membrane of the cell, enters into a single transport pool in the cell, and is actively transported across the serosal plasma membrane. Koefoed-Johnsen and Ussing (1) first proposed a similar theory of sodium transport in the frog skin. Frazier (2) modified their theory in adapting it to the toad bladder.

Frazier and Hammer (3) measured the rates of elution of ^{24}Na from both the serosal and mucosal sides of the bladder, and the results of their experiments were compatible with the above theory. However, by the design of their experiments, they exposed the bladder to ^{24}Na in the elution chambers. It was 7 min before the mucosal chamber was free of the original bath, so that the initial 7 min were lost from the elution curve. Frazier *et al.* defined the sodium transport pool as the fraction of Na in the toad bladder which is labeled with radioactive Na when steady-state conditions have been reached with the isotope label in the mucosal fluid and the serosal fluid virtually free of labeled sodium. The size of this pool was reported as 9.3 μeq/g of tissue water, which calculated to be 7.5 μeq/g of wet tissue based on the average water content these authors reported. On the other hand, the total Na content is reported by these same authors as being 86.9 μeq/g of tissue water.

This paper is a report of experiments which indicate that the sodium transport pool is made up of more than one compartment. Bladder ^{24}Na elution curves have been determined and these show a fast and slow exchange rate evident from 1–18 or more min. During the 0- to 1-min interval there is an elution of ^{24}Na in excess of that expected from determined ^{14}C inulin spaces plus the fast and slow rates observed, suggesting a very fast exchanging compartment. In the experiments the bladders were exposed to ^{24}Na-labeled solutions and subsequently mounted in an eluting chamber after blotting excess bath from the bladder. The elution curve was determined. We have studied only the exchange of the sodium in the transport pool because our interest is in trying to understand more about the intracellular and membrane mechanisms involved in sodium transport.

Materials and Methods. Bufo marinus, ^{14}C inulin sources, the composition of both regular and choline Ringer solution, and details of beta counting have been previously reported (4, 5). Ringer solution containing 60 meq Na/liter was made by mixing calculated volumes of choline and regular Ringer solution. ^{24}Na was supplied by NSEC Division of International Chemical and Nuclear Corp., Pittsburg, Pa. Gamma counting was done on a Tracerlab scaler-spectrometer attached to a well-type scintillation detector. Most counting was to a statistical accuracy of 1.4%. Occasionally, counts with low activity were counted to an accuracy of only 3%.

The specially designed mucosal and serosal elution chambers are shown in Fig. 1. The mucosal chamber is a segment of a 1.8-cm-diameter sphere with a volume of 0.55 cc.

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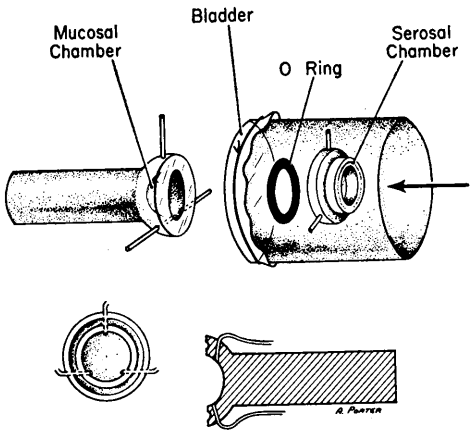

FIG. 1. A drawing showing the essential features of the eluting chambers, and showing the bladder mounted on the plastic tube in the process of being placed between the chambers used for elution. Connecting inflow tubes to chambers have been omitted.

The serosal chamber is a thin cylinder with an internal diameter of 1.6 cm and a volume of 0.50 cc. The rubber O ring is placed on the serosal side of the bladder, which increases the volume of the serosal chamber to 0.85 cc. Each chamber has a single outlet and two inlets. The inlets on the mucosal side are offset so that they neither point toward the center of the chamber nor toward each other. This creates a turbulent flow, which, combined with the use of a smooth curved surface, is credited with producing excellent washout characteristics of the chamber. Less than 1×10^{-5} of a ^{24}Na sample remained in the mucosal chamber after 20 cc of fluid had washed through it when Saran® wrap was in place between two chambers.

Methods. ^{24}Na elution. Bladders were dissected from pithed toads. A hemibladder was split, and a single layer of bladder tissue was stretched over the end of a rigid plastic tube 5 cm in diameter and 7 cm long, with the serosal tissue facing the internal portion of the tube. The mounted bladder was placed with 60 meq Na/liter Ringer solution on the mucosal side and regular Ringer solution on the serosal side, and allowed to sit for 15–60 min. Before exposure to ^{24}Na, the cylinder was removed from the beaker, and the mucosal side of the bladder was blotted free of excess moisture. The cylinder was then immersed in ^{24}Na-labeled (60 meq/liter) Ringer solution for 15 min with the serosal fluid being changed at 10, 12, 13, and 14 min. The serosal solution was collected at 15 min and the mucosal side blotted. Since the bladder was already stretched, it was possible to mount the bladder in 2–4 min on the smaller chambers used for the elution.

After mounting, regular Ringer solution was washed through the mucosal and serosal chambers at a rate of 16.0–20.0 cc/min. Outflow was collected in separate glass test tubes for gamma counting. Flow was interrupted at 0.5- to 1.0-min intervals to change tubes. After 6 min the amount of isotope eluted in a minute was not sufficient for accurate counting, so flow was stopped for 1 min and then resumed for 1 min, giving a 2-min elution period. Still later in the elution, flow was stopped for 2 min and collection made in the subsequent minute, giving a 3-min elution period. The fluid in the collection tubes was diluted to a uniform volume before counting to assure uniform geometry of counting samples. Bladders were checked for leaks both before and after elution.

After elution of the ^{24}Na from the bladder for a total of 18–31 min, flow was stopped, the chamber dismantled, and the eluted portion of the bladder removed, blotted between sheets of filter paper, weighed in a tared beaker for wet weight, dried at 110° for 12 hr, and weighed again for dry weight.

^{24}Na calculation. The quantity of isotope in samples was expressed in counts per minute (cpm) using standard methods of correcting for background and decay. The specific activity (SA) of the sodium in the bath was determined and expressed in cpm/μeq.

The following calculations were carried out for each sample:

$$(\text{cpm/collected sample})/(T \times \text{SA}) = \mu\text{eq Na eluted/min.}$$

T = elution time in minutes per sample.

The natural log of microequivalents eluted per minute was then plotted against time, using the midpoint of the elution period for time. Points were selected beyond 5 min on the basis of their reliability as judged by the

counting data, and these were used to determine the equation for the slow rate. The best fit of a straight line to the data points was determined by the least-squares method of analysis.

The points collected from 1–5 min were then corrected for the effect of the slow rate, and the new points were used to determine the equation of the fast rate using the same techniques. The area under each of these theoretical curves from time 0 to infinity was obtained by appropriate integration and expressed as microequivalents per gram wet weight. All calculations were done on an International Business Machine 1800 computer.

The ^{24}Na that would be present in the extracellular fluid (ECF), on the serosal side of the bladder at the onset of the elution period was calculated on the basis of the following assumptions. The ECF volume was assumed to be 50% of the wet weight (6). It was assumed that the ^{24}Na in the serosal ECF was at equilibrium with the ^{24}Na in the Ringer solution recovered from the serosal side at the end of the exposure period. The ^{24}Na that would be present in the ECF on the mucosal side was similarly determined using values for mucosal ECF as noted below.

^{14}C-labeled inulin experiments. Two different sets of ^{14}C-labeled inulin experiments were done. The rate of elution of the mucosal inulin space from the chamber was determined by exposing the bladders to inulin in Ringer solution for 15 min, and eluting the inulin in a manner identical to that described for ^{24}Na. The ^{14}C inulin was determined by β scintillation counting. The size of this space under various conditions of blotting the bladder was determined by similarly exposing the bladder to labeled inulin in Ringer solution and blotting it free of excess fluid using varying pressures as described in the results section. The bladder was cut free, the ^{14}C inulin was eluted into unlabeled regular Ringer solution, and an aliquot was taken for counting. The wet weight of the bladder was determined.

Results. Elution of ECF from bladder. To determine the rate of elution of a substance in the ECF of the bladder, the bladders exposed to ^{14}C-labeled inulin in Ringer solution on the mucosal side were eluted with regular Ringer solution at a flow rate of 16 cc/min and samples were collected at 0.25-min intervals for 2 min. In three of four bladders more than 99% of the inulin was eluted in 1 min. In the fifth bladder, 5% of the inulin was eluted in the period from 1.00 to 1.25 min, and then less than 1% of the inulin was eluted from 1.25 to 2.00 min.

Determination of mucosal ECF space. The mucosal inulin space is reported to be from 0–16% of wet weight (7). The usual blotting procedure in determining this is to press the bladder firmly between two pieces of filter paper to free it of excess moisture. Since the bladders used in our study were pressed on filter paper with essentially 1–2 cm of water pressure, the inulin space was determined on six bladders which were blotted by this method. The wet weight was determined after conventional firm blotting. The ECF space as percentage of wet weight was 15.4, 16.8, 17.8, 26.7, 30.8, and 39.7, averaging 24.5%. Because these values were higher than published figures, similar determinations were made in which the tissue was blotted by heavy pressure between two sheets of filter paper. This gave values of 4.6, 5.0, 8.5, and 9.3%, which are in agreement with the literature. This indicates that there is a difference in the amount of ECF remaining on the surface of the bladder depending upon the amount of pressure exerted in the blotting process.

Elution of ^{24}Na from the mucosal side. The microequivalents of Na eluted per minute from a typical bladder are plotted on logarithmic scale against time in Fig. 2. These points do not form a single straight line, as would be predicted if the Na were being eluted from a single compartment. After the first minute the curve appears to be made up of at least two rates. The point collected from 0–1 min will be discussed below.

The rates from all experiments are given in Table I. The slow rate averages —0.120, (—0.058 to —0.173) min^{-1} giving a half-time for the average rate of 5.78 min. The

TABLE I. Rate Constants and Size of the Various Compartments of the Toad Bladder.

	Mucosal side					Serosal side				
	Rate constants (min^{-1})		Area under curve (μeq/g wet wt)		0–1 min "extra" Na[a]	Rate constants (min^{-1})		Area under curve (μeq/g wet wt)		0–1 min "extra" Na[a]
Bladder	FM[b]	SM[b]	FM[b]	SM[b]	(μeq/g wet wt)	FS[b]	SS[b]	FS[b]	SS[b]	(μeq/g wet wt)
A	0.723	0.110	8.16	6.87	24.15	[c]	[c]	[c]	[c]	[c]
B	1.024	0.135	4.04	1.32	9.04	0.479	0.285	1.16	15.47	0.639
C	1.133	0.133	8.17	2.71	[d]	0.956	0.053	12.99	2.35	0.746
D	0.816	0.107	5.40	3.24	2.37	0.907	0.036	5.59	5.28	0.430
E	0.788	0.117	6.94	1.54	[d]	0.376	0.133	3.49	2.17	0.484
F	0.758	0.171	6.04	5.58	1.75	1.759	0.098	2.48	3.78	0.320
G	0.774	0.074	6.88	2.66	9.27	0.711	0.098	11.83	1.78	0.652
H	0.725	0.058	8.21	2.29	15.09	0.818	0.137	10.86	2.68	0.308
I	0.701	0.173	3.22	0.56	[d]	0.515	0.039	6.01	0.81	0.218

[a] Extra sodium is that sodium in excess of Na in inulin space, plus calculated fraction eluted in 0–1 min from both fast and slow compartments. See text.

[b] FM and SM are fast and slow mucosal compartments and FS and SS are fast and slow serosal compartments respectively.

[c] In this experiment, activities were too low to complete counting on both mucosal and serosal sides to the accuracy desired. Mucosal samples were to an accuracy of 3% or better, but counting of serosal samples was not completed.

[d] Indicates a negative value.

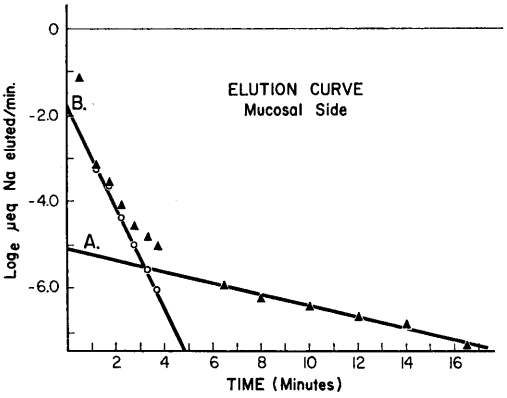

FIG. 2. Elution curve of bladder C on the mucosal side. Each ▲ represents an original data point. Each 0 represents an early point corrected for the effect of the slow rate. The straight line drawn represents the best fit to the data points using the least-squares method of analysis.

fast rate averages —0.827 (—0.701 to —1.133) min^{-1} giving a half-time for the average rate of 0.838 min. The ratio of the Na in the slow compartment to the total in the two compartments varied from 0.182 to 0.457. This is taken as evidence that the slow compartment did not represent an exchange from that portion of the bladder under the O ring of the apparatus, since such a compartment should have a fairly constant ratio to total sodium eluted from the bladder.

Elution of ^{24}Na from the serosal side. When the elution of the radioactivity from the serosal side of the chamber is plotted on a logarithmic scale against time as in Fig. 3, again there is evidence of two rates of elution. The slow rate averages—0.098 (—0.036 to —0.285) min^{-1} giving a half-time of the average rate of 7.09 min. The fast rate averages —0.725 (—0.376 to —1.759) min^{-1} giving a half-time of the average rate of 0.956 min.

Elution from both sides, 0–1 min. It is obvious from the above data that during the first minute of the elution from either side there would be fractions of radioactive sodium in the eluate from three compartments. These three fractions would be the sodium in the inulin space, the sodium eluted in the first minute from the fast compartment, and the sodium eluted in the first minute from the slow compartment, all from the side in question.

As reported in Table I, the sodium eluted exceeded this expected sum in many samples. In the case of the mucosal side, the inulin space was calculated as 24.5% of the determined wet weight, which is the average determined value. The variation of this value was great, and so this fraction would not be estimated accurately for each individual bladder. Using this value, six of nine bladders showed more sodium than would be predicted due to the three fractions described above. Even if 39.7%, the highest value for the inulin space, was used, three of the bladders showed excess sodium. This strongly suggests that there is a very rapidly exchanging compartment which is eluted at a rate such that virtually complete elution is accomplished within the first minute. Further study of this compartment is desirable using a double-label technique in which the Ringer solution is labeled with both ^{24}Na- and ^{14}C-labeled inulin. If this were done and cuts taken of the elution at fractions of a minute, the size and exchange characteristics of the compartments in question could be determined.

On the serosal side a similar calculation using 50% of the wet weight as the size of the serosal inulin space revealed extra sodium in all eight of the bladders reported. A simi-

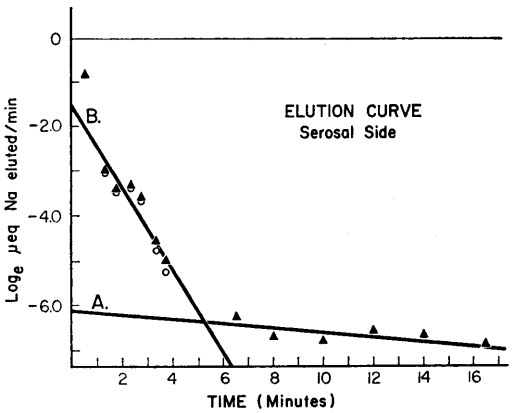

FIG. 3. Elution curve of bladder C on the serosal side. Each ▲ represents original data points. Each 0 represents an early point corrected for the effect of the slow rate. The straight line drawn represents the best fit to the data points using the least-squares method of analysis.

lar variation in the size of the inulin space probably existed. Such an error is not so important here because the concentration of the radioactivity in the serosal bath is low, and hence the quantity of radioactive sodium in this space is small.

Discussion. We were concerned as to whether the phenomenon of two different rates of elution after 1 min was an artifact or not. Frazier and Hammer (3) published curves of elution of radioactive sodium from the toad bladder, two curves from the mucosal and two curves from the serosal sides. These curves began at 7 min after the onset of elution. Rates were obtained from these published curves, by visually drawing a straight line, then calculating the slope of this line. For the mucosal side, the rates were —0.128 and —0.086 min^{-1}; and for the serosal sides, —0.135 and —0.131 min^{-1}. These are in good agreement with the slow rates obtained in the blotted bladders. It is concluded that the blotting did not affect the observed slow rate.

Since these rates agree, the only way that blotting could cause an artifact which gives two rates would be for it to increase the permeability for a short period of time and for the membrane to recover in less than 7 min. Also, since the curves are uniform for each bladder, the recovery process would have to be at a uniform rate. This seems unlikely.

In interpreting the curves, it is important to understand what rate is being measured. First of all we are assuming that the bladder is at a steady-state condition with regard to the sodium in the transport pool. Sufficient time has elapsed from the dissection of the bladder for this to be reasonable. The concentration of Na in the bath was changed from 60 meq Na/liter during exposure to 117 meq/liter during elution. The 60 meq Na/liter Ringer solution was used to gain a higher specific activity of Na in the exposing bath. Frazier *et al.* (7) reported that this does not change the content of the sodium transport pool, so the steady state assumption is still valid.

For simplicity, let us examine what the rate would be if rates from the mucosal and serosal sides of a single compartment were being determined under steady-state conditions. One's first impression regarding this might be misleading. The rate constant seen on one side of the bladder might seem to be the fraction of sodium in the compartment which leaves the compartment through that side per unit time. Under the conditions of the washout experiment, this is not true.

The total sodium content of the compartment is constant. The specific activity of this sodium is changing as the compartment is now exchanging its labeled sodium for sodium in baths which contain no ^{24}Na. This change in the specific activity is the factor which changes the amount of ^{24}Na eluted per unit time from a given side. The rate of change of the specific activity is not a function of the exchange from just one side, but instead is a function of the rate of turnover of sodium in the compartment. This turnover is due to all the exchange which is occurring between the compartment and the two sides. In other words, it can be measured by either the total sodium leaving the compartment per unit time, or the total sodium entering the compartment per unit time. These two totals are, of course, equal, since the sodium content of the compartment is constant in the steady state.

This then means that the rate of change of the quantity of ^{24}Na eluting across one side of the cell is a function of the combined rates of exchange on each side. A corollary of this is that if the ^{24}Na being eluted from both the mucosal and serosal sides is coming from the same compartment, the observed rate of change will be equal.

All of the above three paragraphs can be summarized in mathematical expressions. Let k_m and k_s be rate constants which represent the fraction per unit time of sodium leaving the compartment to the mucosal and serosal sides, respectively. S represents the quantity of sodium in the compartment, and p^* the specific activity of that sodium. It then follows that $dP_m/dt = k_m S p^*$, and $dP_s/dt = k_s S p^*$, where dP_m and dP_s are the quantity of radioactivity appearing in the mucosal and serosal eluate, respectively, in time-interval

dt. Since k_m, k_s, and S, are constants, it is readily seen that the rate obtained is the rate of change of p^*, and would be the same from each side. As shown by Curran, Herrera, and Flanigan (8) this rate is a function of the combined rate at which sodium is leaving the compartment from the mucosal and serosal side. Since the sodium content is at a steady state, the rate of change of p^* is also a function of the rate at which sodium is entering from both sides.

It is evident that both the serosal and mucosal sides show a fast rate of elution of ^{24}Na. It is known that the mucosal to serosal flux in the toad bladder is small and the reverse flux is even smaller. If the fast rates of elution came from a single compartment, this compartment would represent a pathway by which sodium could rapidly cross the bladder in either direction. In order for the flux to be small the following possibilities then exist.

A. The fast rates from the serosal and mucosal sides are not from the same compartment.

B. If one assumes that the rates are from the same compartment, one must evaluate how the flux in both directions could be a small quantity. Let us consider steady-state conditions for the quantity of isotope in this compartment with the label on only the mucosal side. Using the same symbols as above, p^* would be constant. $dP_s/dt = k_s S p^*$ = the mucosal to serosal flux and must be small. Therefore; either,
   1. S can be large, but k_s must be very small in relation to k_m or k_m must be small in relation to k_s; or,
   2. if k_m and k_s are of similar size, then S must be very small.

No other combination of values will give a small flux in both directions if a single compartment is exchanging directly with both sides of the baldder.

The data now allow us to evaluate the possibilities of B-1 or B-2 being true. The amount of sodium eluted from each side in a one-compartment system would total S. This has been determined as described. As can be seen in Table I, this value is large, and so case B-2 above is excluded. The ratios of k_m to k_s are the same as the ratios of the relative amounts of sodium represented by the area under the fast mucosal curve to the area under the fast serosal curve. These ratios are between 4:1 and 1:4 in all of the eight bladders; hence, B-1 possibility is excluded, which rules out the single fast-compartment concept. Therefore, the fast serosal elution curve must come from a different compartment than the fast mucosal elution curve. It is possible that the fast serosal curve represents a compartment on the serosal side of the plasma membrane of the epithelial cell which was back-labeled because the serosal solution was not continually being replaced during the labeling procedure. It could then be a real compartment, but not a part of the sodium transport pool. Experiments exposing the bladder directly in the chamber are planned to clarify this point. Under these conditions, there can be a continuous flow of fluid through the serosal chamber throughout the exposure period.

To evaluate the slow rates of the serosal and mucosal side let us consider a corollary of the facts regarding a single compartment which are presented above. The corollary is of the statement above that "it is readily seen that the rate obtained is the rate of change of p^* and would be the same from each side."

It then follows that if two similar rates are determined on opposite sides of the bladder, one could compare these two rates as a test of whether they represented the elution from the same compartment or from different compartments. If the rates could be shown to be significantly different by accepted statistical techniques, this would be evidence for elution from two different compartments.

In five of eight bladders, the rate constants seen on the two sides differ by more than a factor of 2. These rate constants were derived from data which had the least statistical accuracy because of the slow counting rates. An evaluation of the accuracy of the rate constants will be necessary in order to determine whether they are significantly different.

Experiments will be designed which will give greater accuracy in the determination of these rate constants, and will then give better data to answer the question of whether these are from the same or different compartments.

The "extra" sodium contained in the first-minute elution from both sides indicates that there is sodium contained in some volume of fluid which is greater than the inulin space, and yet elutes very rapidly from the bladder. Functionally, it probably means that the epithelial cell diffusion barrier for Na is different from that for inulin. This is true on both sides of the cell.

The values of this mucosal residual sodium per gram reported in Table I show a wide range. Simple arithmetic makes the reason for this apparent and demonstrates that the size of this compartment must be evaluated in the future by a double-label experiment using both ^{14}C-labeled inulin and radioactive sodium in the mucosal bath. As cited from the literature, the inulin space may vary from 0 to 16% under conditions of firm blotting. Under our conditions of blotting, a film of water may remain on the mucosal side which represents in one case alsmost 40% of the weight of the bladder determined after firm blotting. This value varied from 15.4 to 39.7%. Ten per cent of the bladder would represent 0.1 cc of Ringer per g wet bladder and, with 60 meq Na/liter, this is then 6 μeq/g wet weight of bladder. Thus, the variation in the actual inulin space from the assumed average can account for variation in the size of the "mucosal residual" compartment. Further speculation about these spaces is unjustified on the available data. It seems obvious that these very rapidly exchanging sodium spaces are different from one another on the two sides of the bladder.

It would seem that one would be justified in referring to the following functional spaces or compartments of the toad bladder: (1) the sodium in the inulin space on the mucosal side; (2) the very rapidly exchanging sodium space on the mucosal side; (3) the fast mucosal exchange; (4) slow mucosal exchange; (5) slow serosal exchange; (6) fast serosal exchange; (7) very rapidly exchanging space on the serosal side; (8) serosal ECF space. It seems probable that all of these would contribute to the sodium in the transport pool when measured by counting the bladder at steady-state conditions after exposure with isotope in the mucosal bath, although the contribution of 7 and 8 would be small due to the fact that the fast rate of exchange would keep the specific activity of the sodium in them low. It is probable that the differentiating of compartments 1 and 2, and 7 and 8 has little functional significance. Whether 4 and 5 are two different manifestations of the same compartment remains to be evaluated as discussed in detail above. Although these have been designated as the sodium transport pool in previous work, each will have to be reevaluated to see if it truly plays an important role in sodium transport.

Summary. Elution curves from both the mucosal and serosal sides of the toad bladder indicate that the sodium transport pool is not a single compartment as previously described in the literature. From 1 to 18 or more minutes there is evidence of two rates of elution from each side of the bladder. From 0 to 1 min there is evidence of a larger quantity of Na being eluted than can be expected from the ECF alone. Arguments are presented that there must be at least five compartments and perhaps as many as eight compartments in the sodium transport pool. Two of these compartments would be Na in the serosal and mucosal inulin spaces.

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