Movement of Sodium Across the Mucosal Surface of the Isolated Toad Bladder and its Modification by Vasopressin

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ABSTRACT Studies have been made on the isolated urinary bladder of the toad, *Bufo marinus*, in an attempt to evaluate gradients of chemical activity across the mucosal surfaces of the epithelial cells which would serve to maintain a net movement of sodium from the mucosal medium into the cells. The likelihood of such chemical gradients has been established by the demonstration of lower contents of sodium within the tissue, expressed as microequivalents per gram of tissue water, than of concentrations of sodium in the mucosal medium at all levels of the latter examined. The transepithelial transport of sodium and the sodium content of the tissue were found to increase rapidly with rise in concentration of sodium in the mucosal medium up to values of 30 to 60 meq per liter. Further increase in concentration of the medium above this value failed to induce further stimulation of sodium transport or increase in the sodium content of the tissue. Vasopressin increased the rate of transport of sodium at every concentration of sodium in the mucosal medium without altering this relationship. Although entry of sodium across the mucosal surface of the epithelial cells may be passive it is not by free diffusion but involves some considerable interaction with the mucosal surface of the bladder and constitutes the major determinant of the rate of transepithelial transport of sodium. Vasopressin acts to enhance this initial step in the transport of sodium.

INTRODUCTION

The preceding paper (1) considered possible electrical driving forces for net movements of sodium across the mucosal surface of the single layer of epithelial cells which line the urinary side of the isolated toad bladder. Direct measurements indicate that electrical gradients in the spontaneously active bladder actually oppose net entry of sodium whereas in the short-circuited preparation a small electrical driving force is present.

The purpose of the present paper is to determine whether gradients of chemical concentration can be implicated as the driving force for the initial entry of sodium across the mucosal boundary of this epithelial layer and to localize the site of the large stimulatory effect of neurohypophyseal hormones on sodium transport (2). The results indicate that gradients of concentration which favor net entry of sodium across the mucosal surface into the cells exist but that the movement of sodium across this cell surface is not by free diffusion but involves a considerable interaction with the membrane. The stimulatory effect of neurohypophyseal hormones is attributable to an enhancement of this initial step in transepithelial transport of sodium.

METHODS

Studies were done using the urinary bladder of the toad, *Bufo marinus*, *in vitro* (2). The bathing media have all been previously described (3). Osmolality of the solutions was checked with a Fiske osmometer. Sodium concentrations of media and tissues were determined with a Baird atomic flame photometer (Cambridge, Massachusetts).

The sodium content in the bladder involved in the active transport of sodium across this tissue ("the active sodium transport pool") was determined by two separate methods: Na^{22} or Na^{24} was added to the Ringer's solution bathing the mucosal surface of the bladder which was mounted between the two conventional lucite half-chambers used in short-circuit current measurements (4). All the sodium was excluded from the serosal bathing medium through the use of choline Ringer's solution on this side of the bladder. Short-circuit current measurements were made for a period of 60 minutes after the isotope was added. The half-bladder was then quickly removed from the chamber, blotted carefully on Whatman No. 54 filter paper, the wet weight obtained by weighing in tared tubes, and the radioactivity of the tissue counted together with that of a sample of the mucosal bathing medium in a Packard auto-gamma spectrometer (Packard Instruments Co., LaGrange, Illinois). The tissue was subsequently dried 24 hours at 95°C and reweighed to obtain the dry weight of the tissue. This method of handling the tissue has been shown to yield reproducible values for tissue water content of 80.8 ± 1 per cent (S.D.) of wet weight and to result in only small residual contamination of the mucosal surface with adherent bathing medium. The latter was indicated by the finding that C^{14}-inulin added just to the mucosal medium came into diffusion equilibrium with some 7 per cent of tissue water on the average, range 0 to 16 per cent; the figure was unaffected by neurohypophyseal hormones (5).

From the counts in the tissue, those in the mucosal medium, and the known sodium concentration of the latter the tissue sodium content per gram of tissue water could be estimated.

$$[Na]_t = \frac{(\text{Tissue counts})\ [Na]_m}{(\text{Medium counts})}$$

in which:

$[Na]_t$ = microequivalents of sodium per gram of tissue water

$[Na]_m$ = microequivalents of sodium per milliliter of mucosal medium

Tissue counts = counts per minute per gram of tissue water

Medium counts = counts per minute per milliliter of mucosal medium.

This method ensures that all the estimated sodium must have entered the tissue through its mucosal surface. However, comparison of the pool determined in this manner with the total sodium content measured directly by flame photometry on a nitric acid eluate of the dried tissue revealed that the isotopic method measured only 7.6 μeq of sodium per gram of tissue water as compared with 14.6 μeq of sodium per

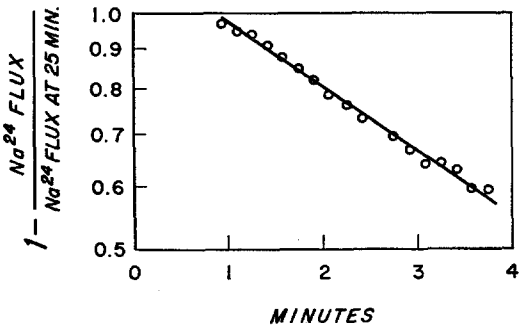

FIGURE 1. The rate of appearance of radioactivity in the serosal bathing medium when Na^{24} was abruptly added to the mucosal medium. A straight line was fitted by inspection and the half-time for establishment of a constant specific activity of the sodium crossing the toad bladder found to be less than 4 minutes. The linearity of the plot is evidence that isotopic sodium is mixing with a single pool. The delay in the initial appearance of the counts in the serosal medium represents the time required for the passage of the labeled solution from a shielded reservoir at a distance from the chamber.

gram of tissue water from direct analysis. The mean difference in this series of fifty-one observations was 7.0 and the standard error of the mean difference ± 0.43 μeq per gram tissue water. The component of tissue sodium which was not in exchange equilibrium with radioactive sodium from the mucosal medium comes promptly into equilibrium when the radioactive sodium is added also to the serosal medium. This difference therefore represents sodium which is not in the active transport pool and may well be within the connective tissue or smooth muscle cells on the serosal side of the bladder.

In order to determine whether the time of exposure was sufficient to uniformly label the active sodium transport pool as well as to confirm that all the sodium which entered through the mucosal surface in these isotopic measurements was involved in the active transport pool, the size of the latter was estimated kinetically by the second method. The conventional lucite half-chambers were modified so that each surface of the mounted half-bladder could be exposed to a continuous flow of bathing medium from a reservoir. The outflow of each half-chamber could be col-

lected on long strips of Whatman No. 1 filter paper arranged to move at a constant rate of 115 cm per minute past the chamber outlets. The strips of paper could then be cut into lengths representing a known brief time interval, placed in test tubes, and the activity of Na^{24} counted in the Packard auto-gamma spectrometer. The surface area of tissue exposed in such a flow chamber was 3.1 cm^2, the volume of each half-chamber was 0.7 ml, and the rate of flow was 4 to 6 ml per minute.

With a half-bladder mounted in such a flow chamber a steady state of sodium transport was obtained as evidenced by a constant short-circuit current while a constant flow of Ringer's solution passed each surface of the bladder. The mucosal bathing medium was then abruptly switched to one of identical composition but with the sodium labeled with tracer amounts of Na^{24}, thus maintaining a fixed specific activity of Na^{24} in contact with the mucosal surface during the remainder of the experiment. The effluent from the serosal medium was caught on the moving

TABLE I

CORRESPONDENCE BETWEEN SHORT-CIRCUIT CURRENT AND MUCOSAL TO SEROSAL FLUX OF SODIUM WITH CHOLINE RINGER'S SOLUTION BATHING SEROSAL SURFACE

Mucosal Na Concentration	No. of 30 min. periods	Short-circuit current	Na flux (M→ S)	Δ	s. e. of mean difference
meq/liter		*μa*	*μa*	*μa*	*μa*
5	8	136	104	+32	23.5
20	8	236	234	+ 2	6.8
60	31	294	306	−12	9.7
114	20	368	392	−24	12.7

Simultaneous measurements of short-circuit current and of unidirectional sodium flux measured from mucosal to serosal surface with Na^{22} or Na^{24}.
Cross-sectional area of chamber = 7.07 $cm.^2$
15 ml of sodium-containing Ringer's solution bathing mucosal surface and 15 ml sodium-free Ringer's solution on serosal side.

strip of filter paper, dried, placed in tubes, and counted as described. The rate of appearance of counts on the serosal side could be plotted as indicated in Fig. 1. The half-time for this process was found in seven determinations to vary from 1.0 to 4.6 minutes, thus establishing that the 60 minutes allowed by the first method was more than ample time for complete equilibration of mucosal sodium with that in the active transport pool. Furthermore, from the half-time for appearance, the steady state value, and the fact that some 90 per cent of the unidirectional flux of sodium from the mucosal to serosal surfaces of this tissue moves by way of the active transport pathways (6), one can calculate the size of the active sodium transport pool in this epithelium. The method is analogous to that recently reported by Hoshiko and Ussing (7). The results obtained by this method in seven measurements corresponded satisfactorily with those by the first method and this justifies our use of the simpler first method in these studies.

The short-circuit current was measured in these studies in order to obtain the rate of sodium movement through the "active transport pathways" under repro-

ducible and standard conditions. Since the short-circuit current has been measured with choline-Ringer's solution bathing the serosal surface of the bladder, that is with a chemical gradient for sodium across the thickness of the bladder wall, it becomes essential to know: (*a*) whether the short-circuit current still equals the net transfer of sodium from mucosal to serosal surfaces, and (*b*) whether sodium still follows the active transport pathways under these conditions. A comparison of short-circuit current and simultaneously determined unidirectional sodium flux is presented in Table I. The good agreement between these two measurements constitutes our justification for using the short-circuit current as the measurement of net sodium flux under these circumstances.

Although Table I indicates good agreement between net sodium flux and short-circuit current, it does not establish that all the sodium moves through the active transport pathways. Sodium leaking through low resistance channels in the membrane might be included in our estimates of active transport. To resolve this possibility use was made of the fact that active transport of sodium is dependent upon the presence of potassium in the serosal bathing medium. In the absence of potassium sodium transport is halted (8, 9). Since omission of potassium from the choline Ringer's solution bathing the serosal surface likewise reduced the short-circuit current nearly to zero, it may be assumed that the short-circuit current measured in choline Ringer's solution in the presence of potassium does in fact involve sodium moving through the usual active transport pathways.

The Na^{24} used was prepared on the atomic reactor of the Massachusetts Institute of Technology and supplied by the Iso/Serve Co., Boston.

RESULTS

Transport of Sodium from Very Low Concentration in Mucosal Bathing Medium

With the usual amphibian Ringer's solution bathing each surface of the toad bladder, mean tissue concentration of sodium was 86.9 ± 3.6 (S.E.M., $n = 20$) μeq per gram tissue water. The concentration of sodium in the Ringer's solution was 114 μeq per ml. In this situation, therefore, a concentration gradient exists which could serve as the driving force for net entry of sodium through the mucosal surface of the toad bladder. However, a more rigorous test for the presence of concentration gradients may be made at low concentrations of sodium in the mucosal medium.

To test for active transport of sodium from low concentrations in the mucosal bathing medium choline Ringer's solution replaced the usual Ringer's solution bathing that surface. In five experiments with concentrations of sodium less than 1.0 meq per liter, small short-circuit currents and transmembrane electrical potentials could be measured which had the usual orientation—serosal surface positive to mucosal surface. One such experiment is presented in Fig. 2. In spite of an initial mucosal concentration of sodium of 0.3 meq

per liter a short-circuit current was measured which was closely approximated by the unidirectional transmembrane sodium flux from mucosal to serosal surface simultaneously determined with Na^{22}. After 30 minutes of observation sufficient sodium was added to increase the concentration to 1.70 meq per liter. This resulted in a stepwise increase in transmembrane electrical potential, short-circuit current, and unidirectional flux of sodium from mucosal to serosal surface suggesting that the values measured even at the initial lower concentrations did in fact result from sodium transport. Furthermore, additon of vasopressin produced its usual specific stimulatory effect on potential, short-circuit current, and sodium flux (6). The low rates of net sodium transport in these experiments and the asymmetrical ion distributions prevent accurate double isotope flux measurements to establish active sodium transport (4). The evidence just presented, however, together with the established fact that this tissue does actively transport sodium (2) supports the contention that such active transport may proceed from concentrations in the mucosal medium of less than 0.5 meq per liter.

The Tissue Sodium Pool at Low Mucosal Sodium Concentrations

The evaluation of existing electrical forces across the mucosal surface of the cells indicated that they would make a small contribution to the entry of sodium at this surface in the short-circuited preparation at low concentrations of sodium in the mucosal medium (1). To learn whether concentration gradients were also operative the sodium content in the active transport pool was determined with isotope added to the mucosal medium while all the sodium in the serosal bathing medium was replaced by a choline Ringer's solution, as described in Methods. This latter measure insured that all sodium within the epithelium entered from the mucosal side and that the active transport pool was not underestimated by an unknown and possibly variable amount of sodium entering from the serosal side. All measurements shown in Table II were made with vasopressin in the bathing medium. Table II indicates that both at the usual concentrations of sodium in the mucosal medumi and at the lowest values studied, the sodium content within the tissue, expressed as microequivalents per gram of tissue water, was lower than the sodium concentration in the mucosal medium. The differences in several instances are small and if the values were corrected to concentrations in non-inulin space water of the tissue, which is 49.5 $\pm$ 4.4 (S.E.M., $n = 8$) per cent of tissue water under these circumstances, values slightly higher than the concentration in mucosal medium would be obtained. However, at these very low concentrations, the possible small electrical forces discussed (1), adsorbed radioactive sodium on the mucosal surface, and inequalities in activity of intracellular sodium would all contribute to an erroneously high estimation of the sodium

pool. We therefore prefer to take into account the definite trend for the pool content to decrease with decreasing concentrations of sodium in the mucosal medium and to consider the uncorrected results as presented in Table II. From this we conclude that, although nothing is yet known about the dis-

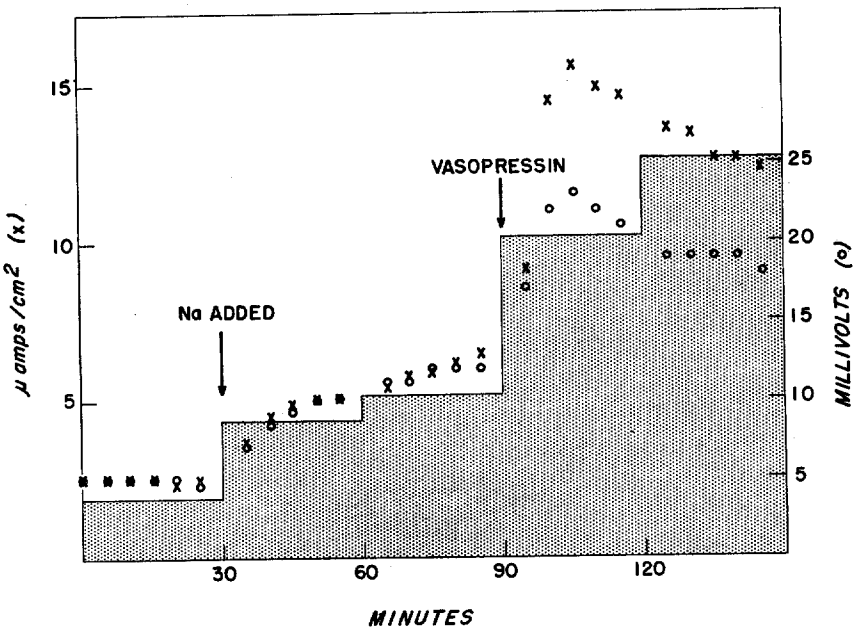

FIGURE 2. Demonstration of equality of short-circuit current and isotopic sodium flux with low concentrations of sodium bathing the mucosal surface of the bladder. The hatched area represents the mucosal to serosal flux of sodium measured isotopically and the small crosses are the short-circuit current. Both values are expressed as microamperes per cm^2 on the left hand ordinate. The spontaneous transmembrane potential is charted as millivolts (scale to right) and indicated by open circles. The sodium concentration initially was 0.3 meq per liter on the mucosal side and a small short-circuit current and unidirectional sodium flux were measured. Increasing the mucosal sodium concentration to 1.70 meq per liter enhanced sodium flux, current, and potential. These parameters showed the characteristic augmentation when vasopressin was added to the serosal medium (6).

tribution of the tissue sodium within the cells, the likelihood of a concentration gradient favoring entry of sodium from mucosal medium into the tissue is established and that this step may be regarded as passive.

The Dependence of Sodium Transport on the Concentration of Sodium in the Mucosal Medium

In five experiments using the flow chamber the short-circuit current, as the measure of active sodium transport (2), was determined as a function of the

concentration of sodium in the mucosal medium. The desired sodium concentration was obtained by diluting the usual sodium Ringer's solution with known volumes of sodium-free choline Ringer's solution. After each change of concentration sufficient time was allowed for the short-circuit current to achieve stable values. Measurements were made first in the absence of vasopressin and then repeated in the same membrane but with vasopressin added to the serosal bathing medium. Fig. 3 shows one such experiment. Of importance to the present study are: (*a*) the initial rapid rise in rate of sodium transport as the concentration of sodium in the mucosal medium is increased with attainment of a maximal rate of sodium transport independent of further elevation of the sodium concentration, and (*b*) the similarity of the shape of the curve in the presence of vasopressin but with the transport rate higher for every concentration of sodium in the medium. Both without and with the hormone present the relationship of active transport to the concentration of sodium in the mucosal medium exhibits typical saturation kinetics with maximal values attained at sodium concentrations of 30 to 60 meq per liter in the mucosal medium. Such a relationship had been previously demonstrated for the unstimulated frog skin by Ussing (10), Kirschner (11), and Snell (12).

The Dependence of the Sodium Transport Pool on the Concentration of Sodium in the Mucosal Medium

Two possibilities suggest themselves to account for the saturation kinetics exhibited by the transepithelial transport of sodium just demonstrated. Either the active extrusion of sodium through the serosal surface could be rate-limiting with passive entry through the mucosal surface by simple diffusion or the mucosal entry could be limiting. These two possibilities would distinctly and characteristically affect the sodium pool within the tissue. In the former case the pool of tissue sodium should increase with the concentration of sodium in the mucosal medium once the serosal extrusion had saturated. On the other hand, if mucosal entry of sodium were limiting its transepithelial transport, the size of the tissue pool should level off with increasing concentrations of sodium in the mucosal medium.

To test these two possibilities the active transport pool was determined as a function of concentration of sodium in the mucosal medium, as described. The results of thirty-one paired experiments are plotted in Fig. 4. Because of variability of the tissue sodium pool from one bladder to another but the fair agreement between values obtained on paired bladder halves, all measurements in Fig. 4 were obtained from paired experiments utilizing the two halves of one bladder. Both halves received identical treatment except that the concentration of sodium in the mucosal bathing medium was always 60 meq per

TABLE II

THE TISSUE SODIUM CONTENT AS A FUNCTION OF THE MUCOSAL SODIUM CONCENTRATION

Concentration in mucosal medium	Tissue content	No. of measurements
μeq/ml	*μeq/gm tissue water*	
114	9.3±1.3 (S.E.M.)	10
60	9.3±1.3 "	10
20	5.6±1.1 "	8
5	2.6±0.32 "	8
1.18	0.64	1
0.98	0.42	1
0.61	0.59	1
0.45	0.43	1
0.40	0.20	1

Mucosal medium was Ringer's solution diluted with sodium-free choline Ringer's solution to obtain the indicated concentrations of sodium.
Serosal medium was a sodium-free choline Ringer's solution.
The sodium pool was estimated with radioactive sodium added to the mucosal medium, as described in Methods.
Chamber area, 7.07 cm^2. Volume of medium bathing each surface, 20 ml. Duration of incubation, 60 minutes. Vasopressin was present in all experiments.

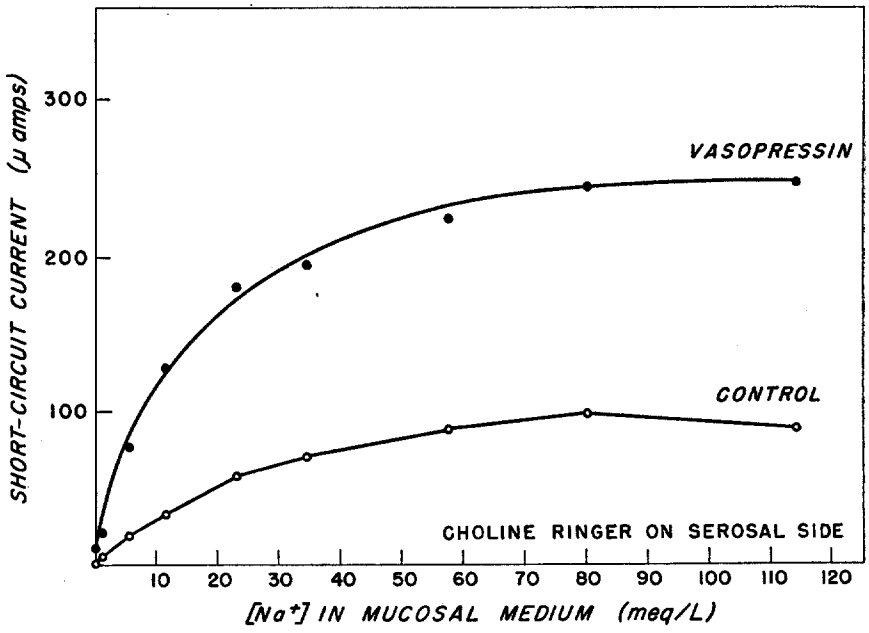

FIGURE 3. The dependence of sodium transport on the concentration of sodium in the mucosal bathing medium in the presence and absence of vasopressin. Measurements of short-circuit current were made in the same bladder half first in absence of vasopressin and then again in its presence.

liter for one-half while the other half was exposed to concentrations of <1, 5, 20, or 114 meq per liter. The ordinate in Fig. 4 is the pool size found at these concentrations expressed as per cent of that found simultaneously in the control half-bladder exposed to 60 meq of sodium per liter. Some of the data of Table II are used in this figure.

The shape of the curve in Fig. 4 mimics that of Fig. 3, clearly indicating that the mucosal entry of sodium into the tissue imposes the major limitation

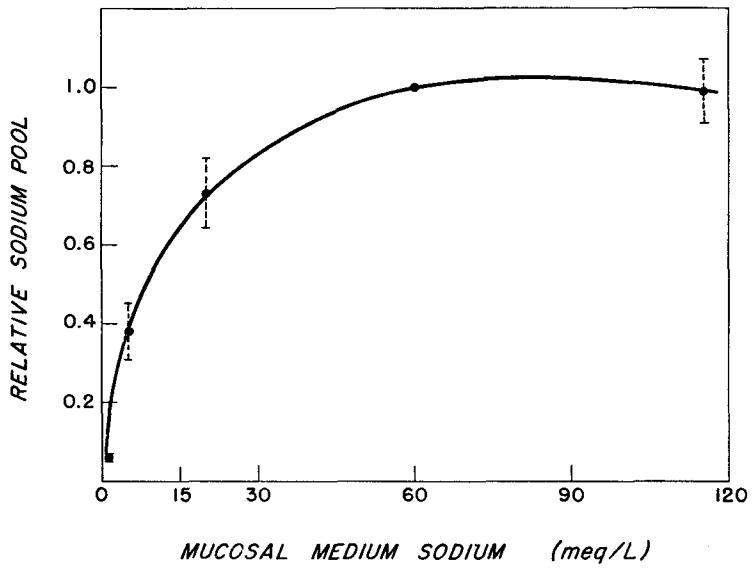

FIGURE 4. The dependence of the tissue pool of sodium on the concentration of sodium in the mucosal bathing medium. Measurements were all made in the presence of vasopressin on paired bladder halves. One bladder half was exposed in each instance to 60 meq of sodium per liter in the mucosal bathing medium while the other half was exposed to <1, 5, 20, or 114 meq of sodium per liter. The ordinate is the ratio of the sodium pool found in the one bladder half to that obtained for the paired half exposed to 60 meq of sodium per liter.

on the transepithelial transport of sodium. This finding demonstrates that though entry of sodium across the mucosal surface may be passive with the sodium moving down its concentration gradient, nevertheless, it cannot be by free diffusion but must involve a considerable interaction of the sodium ions with some component of the mucosal cell membrane.

The Site of the Action of Vasopressin to Enhance Active Sodium Transport

The demonstration that mucosal entry of sodium imposes the major limitation on the transepithelial transport of sodium suggests that vasopressin would act on this step to enhance active sodium transport in this tissue. However,

a direct evaluation of the site of action can be made now that the driving forces for the entry of sodium into the epithelium have been characterized. As previously demonstrated for urea (5) and water (13, 14) the action of this hormone can be evaluated in terms of two permeability barriers, tentatively identified as the plasma membrane on the mucosal and serosal surfaces of the single layer of mucosal cells (5, 15). If the hormone augments the mucosal permeability to sodium the rate of entry of sodium into the pool should be

TABLE III

THE EFFECT OF VASOPRESSIN ON SODIUM TRANSPORT AND THE TISSUE POOL OF SODIUM IN THE TOAD BLADDER

Experiment	Sodium transport Vasopressin		Sodium pool Vasopressin	
	Absent	Present	Absent	Present
	μa/cm²		*μeq/gm tissue water*	
1	18	47	9.7	15.3
2	61	99	11.4	15.3
3	6	22	7.9	19.6
4	5	32	8.9	19.1
5	22	62	7.3	13.1
6	20	50	5.9	9.3
7	26	53	5.9	8.6
8	15	57	8.8	11.1
			Mean 8.2	13.9
			$\Delta = 5.7 \pm 1.3$ (S.E.M.)	
			($p < 0.01$)	

Mucosal medium was Ringer's solution with a concentration of sodium of 114 meq per liter. Serosal medium was a sodium-free choline Ringer's solution.

The sodium pool was estimated with radioactive sodium added to the mucosal medium, as described in Methods.

Chamber area, 7.07 cm.² Volume of medium bathing each surface, 20 ml. Duration of incubation 60 minutes.

Each experiment includes measurements on paired bladder halves treated identically except for presence of vasopressin (25 milliunits per ml) added to serosal medium of one.

enhanced and the size of the sodium pool in the tissue should be increased. If the action of the hormone were to accelerate the extrusion of sodium through the serosal surface then the size of the sodium pool would be decreased in the presence of the hormone. When paired experiments were done on the two halves of one bladder, one half exposed to vasopressin and the other serving as the control, the results in Table III were obtained. The stimulatory effect of vasopressin on active sodium transport is indicated by the larger short-circuit current observed in every instance in the hormone-treated bladder half. In every instance also the tissue sodium pool was found to be larger in the presence of vasopressin. This establishes that the stimulatory effect of vaso-

pressin on active sodium transport is attributable to an action on the mucosal surface of the tissue. This mucosal locus of action is similar to that previously demonstrated for the enhancing effect of vasopressin on permeability to water (13, 14) and urea (5).

DISCUSSION

The model for the transepithelial sodium transport system of frog skin proposed by Koefoed-Johnsen and Ussing (16) includes the passive entry of sodium through the outwardly oriented cell membrane. Both the electrical potentials (1) and chemical gradients of sodium across the corresponding mucosal cell surface of the toad bladder are in accord with such a proposal. The present study, however, demonstrates that, although the initial entry of sodium into the transporting epithelium may be passive, it is a complex step involving interaction of sodium with the membrane. Our results also require that the extrusion of sodium from the epithelial cells into the serosal medium be an active process capable of surmounting opposing chemical and electrical gradients.

In the present investigation an attempt has been made to separate events at the mucosal from those at the serosal surface for purposes of study. It has been demonstrated that the mucosal surface is the major permeability barrier in this tissue to water and to most small solutes (5, 15, 17). That the sodium ions should have a special mode of entry through the mucosal surface is in keeping with the fact that active sodium transport is a major function of this tissue. Increasing the permeability to sodium at the mucosal surface would serve to accelerate reabsorption of sodium from urine to body fluids while at the same time keeping the free energy expenditure per ion transported at the serosal surface minimal. In spite of this facilitated entry, however, the results indicate that this initial step imposes the major limitation to transepithelial transport even in the presence of vasopressin.

Although the intention of this study was to examine the driving forces for the mucosal entry of sodium, it is evident that some information regarding the kinetics of the serosal extrusion of sodium is implicit in the results. A relationship between the short-circuit current and the active transport pool can be obtained by combining the data from Figs. 3 and 4 so as to eliminate the term for mucosal sodium concentration. The resulting expression indicates that sodium transport increases more rapidly than does the size of the transport pool. This demonstrates that the kinetics of active sodium extrusion exert some regulatory effect on the size of the active transport pool. The failure of sodium transport to increase, however, when the mucosal concentration exceeds 60 meq per liter indicates that the major regulatory step resides in the process of mucosal entry of sodium.

Vasopressin was found to increase both the rate of transepithelial transport of sodium and the content of sodium within the tissue. Because the tissue content of sodium was increased despite a more rapid serosal extrusion of sodium, the major action of vasopressin must be to enhance the mucosal entry of sodium.

The assumption has been tacitly made hitherto that the sodium in transit from mucosal to serosal surfaces of the bladder does in fact pass through the mucosal cells rather than follow some intercellular route. The measurements of the size and kinetics of the active transport pool of sodium made by Hoshiko and Ussing (7) indicated to them that this sodium lies within the epithelial cells. The pool sizes determined in the present study would require an intercellular space (not penetrated by inulin) equal to approximately one-sixth of the total water in the non-inulin space of the tissue, if the concentration of sodium in such an hypothetical space were equal to that in the mucosal medium. There is no basis for such a space morphologically. (Electron microscopic studies reveal a very tight apposition of the mucosal plasma membranes of the epithelial cells and closely interdigitating lateral plasma membranes (18)). Nor is there functional evidence for such a space. (Transepithelial permeability to small solutes is generally very low (14).) It, therefore, seems most reasonable to conclude, in accord with Hoshiko and Ussing from their studies with frog skin (7), that sodium enters the epithelium across the plasma membrane at the mucosal surface, traverses cytoplasm, and is extruded across the opposite serosal plasma membrane.

In discussing the intracellular sodium pool we have specifically avoided the term "concentration" but rather adhered to "content per gram of tissue water." At the present time there is no information regarding the distribution of sodium within the mucosal layer of cells. However, if the sodium pool were in fact relegated to only a small fraction of the intracellular volume, so that the assumed chemical gradients did not exist, then it would be most unusual for the intracellular content to be as dependent upon the mucosal concentration of sodium as has in fact been found. Furthermore, compartmentalizing the intracellular sodium would create the further serious problem of having to explain how the concentration in the remainder of the cell was kept so low as to give the low average intracellular concentrations observed. Since cell membranes are generally permeable to sodium, at least to some degree, such compartmentalization would seem to require an extensive system for intracytoplasmic transport of sodium and markedly complicate the situation both for the cell and the investigator! In the present state of knowledge, therefore, it seems simpler to assume that the activity of intracellular sodium is uniform. This is equivalent to postulating that no separate channels for transepithelial transport of sodium exist and that the problems of explaining how the intracellular sodium concentration within the epithelial cells is kept low and how

the transepithelial sodium transport is accomplished are intimately and indivisibly linked.

Since we have shown that the relationship in Fig. 3 of sodium transport to concentration of sodium in the mucosal medium results from interaction of sodium with the mucosal surface one might apply an analysis to these results similar to the formulation by Michaelis and Menten. In fact the double reciprocal plot of Lineweaver and Burke (19) of the results in our five experiments yields two straight lines in each experiment with distinctly different intercepts on the ordinate; values obtained before exposure to vasopressin fall on the upper line with steeper slope. The average value for the Michaelis constant, K_m, was 20 mM in the absence of vasopressin and 21 in its presence, and the value of V was, of course, considerably greater in the presence of the hormone. Such an analysis applied to the present problem unfortunately fails to yield unique information regarding the system. Thus either a greater number of interacting sites operating in parallel in the presence of the hormone or a more rapid turnover of a fixed number of interacting sites would be compatible with the results.

The locus of action of vasopressin on the mucosal permeability barrier of this tissue had been previously postulated before the complete evidence presented in these two papers was available (13). As the site of action seems to be the same for the increased permeability of the tissue to water (13, 14) and urea (5) as well as to sodium some single unifying action of the hormone on the mucosal barrier must be sought.

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