The Kinetics of Sodium

Transport in the Toad Bladder

II. *Dual effects of vasopressin*

ARTHUR L. FINN

From the Departments of Physiology and Medicine, Yale University School of Medicine, New Haven, Connecticut 06510. Dr. Finn's present address is Department of Medicine, School of Medicine, University of North Carolina, Chapel Hill, North Carolina 27514.

ABSTRACT   In the accompanying paper, a compartmental model for the toad bladder sodium transport system was developed. In the present paper, the model is tested by determining the effects of antidiuretic hormone on the pools and fluxes. It is shown that this hormone affects only that sodium pool previously designated as the transport pool, and that the effects are on two separate sites. In the first place, the hormone stimulates entry at the mucosal side of the transport compartment, and by this means brings about an increase in the amount of sodium contained in the compartment. Second, the hormone has a distinct stimulatory effect on the rate coefficient for efflux across the serosal boundary, the pump rate coefficient. Evidence is presented that under control conditions, the pump rate coefficient is a decreasing function of the pool size, a characteristic feature of a saturating system. Therefore, the effect of vasopressin in increasing both the pool size and the pump rate coefficient must be construed as a direct effect on the pump, and not one which is secondary to the increase in the pool size. Furthermore, it is shown that the effect of the hormone on the sodium pump is not dependent on the presence of sodium in the serosal medium.

In the accompanying paper (Finn and Rockoff, 1971) it was shown that it is possible to represent toad bladder sodium content by a compartmental model. It was found that a system containing two noncommunicating tissue compartments satisfied the data obtained from the washout of radioactive sodium, and that one of these compartments was quite likely to be the transport pool. However, in order to establish more firmly that such a model is biologically relevant, it is necessary to demonstrate that biological perturbations affect the system in a consistent and reasonable manner. In the present paper, we explore the effects of antidiuretic hormone on these transport parameters. In addition, some of the effects of changes in the sodium concentration in the media will be examined.

349

It has been shown previously by others (Leaf et al., 1958; Orloff and Handler, 1962; Leaf, 1966) that vasopressin increases sodium transport from the mucosal to the serosal surface of the bladder. There is considerable indirect evidence that a site of action of this substance is on the mucosal-facing side of the epithelial cells (Civan et al., 1966; Civan and Frazier, 1968; Frazier et al., 1962). There is also indirect evidence (Morel and Bastide, 1965; Finn, 1968; Lipton and Edelman, 1969) that vasopressin effects an increase in the activity of the sodium pump as well. In the present paper, it will be shown that vasopressin indeed has a direct stimulatory effect on both the passive entry and the active exit steps and that only the sodium compartment previously designated as the transport pool is affected by the hormone.

MATERIALS AND METHODS

Bufo marinus, obtained from the Pet Farm, Miami, Florida, were used. The Ringer solution was composed, in millimoles per liter, of NaCl, 109, KCl, 2.5, $CaCl_2$, 1, $NaHCO_3$, 2.38, and glucose 5.5. It was gassed with air, and had a pH of 7.8. In some studies choline (recrystallized from 95% alcohol) was substituted for sodium on a mole-for-mole basis.

The toads were pithed, the bladder excised and mounted in the chamber as previously described (Finn and Rockoff, 1971). Following the establishment of a constant short-circuit current, ^{24}Na was added to the mucosal medium, and allowed to remain there until a steady state for tracer was obtained. Washout was then performed as described in the accompanying paper. Subsequently, tracer was again added and vasopressin was added to the serosal bath (final concentration, 50 mU/ml). At the time of maximal increase in short-circuit current (about 15–25 min after vasopressin was added), the washout was begun. In four of the seven studies performed in normal Ringer solution, the experiments were performed in reverse order; i.e., the vasopressin study was done first. In those experiments, the vasopressin was washed off with hormone-free Ringer solution for at least 1 hr before adding tracer for the second washout.

RESULTS

The results of the vasopressin studies on bladders mounted in normal Ringer solution are shown in Table I. In each study, vasopressin effected an increase in the short-circuit current, the mean increment being 95%. Note that there is a significant increase in J_{1M}, the influx from the mucosal medium into the transport pool, and k_{s1}, the rate coefficient for the efflux from the pool to the serosal medium, the "pump rate constant." Values for the pool and the other unidirectional fluxes are not given, since calculation of these values, as shown in the accompanying paper (Finn and Rockoff, 1971), requires a steady state for total sodium. Since the short-circuit current is not stable during the wash period (as the effect of vasopressin is waning despite the

TABLE I

EFFECTS OF ADH ON
THE TRANSPORT POOL

	k_{M1}		k_{S1}		J_{1M}		$P_1(0)/P_M(0) \times 100$	
	Control	ADH	Control	ADH	Control	ADH	Control	ADH
1	0.206	0.195	0.055	0.094	2.76	4.24	0.566	0.839
2*	0.129	0.343	0.032	0.152	0.83	5.39	0.298	0.629
3*	0.380	0.296	0.031	0.055	2.79	2.43	0.402	0.409
4*	0.320	0.296	0.013	0.023	3.86	4.76	0.287	0.378
5	0.471	0.192	0.028	0.064	5.83	12.48	0.594	1.585
6*	0.169	0.066	0.109	0.064	1.74	2.70	0.664	1.493
7	0.350	0.408	0.008	0.033	5.63	9.42	1.537	2.016
Mean	0.289	0.257	0.027	0.069	3.35	5.92	0.621	1.050
±	±	±	±	±	±	±	±	±
SEM	0.047	0.043	0.005	0.016	0.71	1.40	0.162	0.244
Δ	−0.032		0.043		2.57		0.428	
±	±		±		±		±	
SEM	0.057		0.013		0.94		0.138	
p	N.S.		<0.02		<0.05		<0.02	

Rate coefficients are expressed in min^{-1}, and the flux J_{1M} is expressed in $\mu Eq \cdot min^{-1} \cdot 100$ mg dry weight^{-1}. N. S. indicates not significant.
* In these experiments, the vasopressin study was done first.

continued presence of the hormone) this may not be a valid assumption. On the other hand, calculation of the amount of tracer present in the transport pool at the start of the wash, $P_1(0)$, requires a steady state for tracer (which is present or very nearly so), but not for total sodium. One may therefore calculate the relative amount of isotope in the pool by comparing the computer-determined values of $P_1(0)$ with the amount of tracer present in the loading solution, $P_M(0)$. Definitions and symbols are as in the accompanying paper (Finn and Rockoff, 1971), and are shown in Fig. 1.

The large increase in the influx into the pool might be expected to increase the size of the pool, since the fluxes at the serosal side are so small in comparison. As the table shows, the transport pool is increased by the hormone by about a factor of two. It is possible that this increase in the pool size is the cause of the increase in the pump rate coefficient, although one would predict a fall in the latter if the transport system is a saturating one.

There is, in fact, a suggestion from the data in Table I that saturation occurs, since the wide variation in the values of k_{S1} appears to adhere to a pattern, namely, that the higher the pool, the lower the rate coefficient for the pump. In order to demonstrate this more clearly, studies were carried out in which the sodium concentration in the media was changed. Each bladder was studied at least twice. As shown in Table II, it is clear that in any given bladder there is a fall in the pump rate constant as the pool rises,
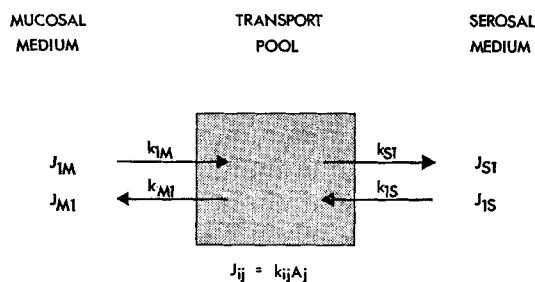
FIGURE 1. Model of the transport pool. J_{ij}, sodium flux into compartment i from compartment j. k_{ij}, rate coefficient. A_j, total sodium in compartment j. M, mucosal medium. S, serosal medium. 1, transport compartment.

TABLE II

EFFECTS OF CHANGING
AMBIENT SODIUM CONCENTRATION

Bladder No.	Sodium concentration	J_{1M}	J_{M1}	J_{S1}	J_{1S}	Pool	k_{M1}	k_{S1}	SCC
	mM								
1	111	0.715	0.606	0.278	0.169	5.20	0.116	0.053	9.0
1	10	0.103	0.078	0.073	0.048	0.68	0.114	0.107	2.1
2	10	0.891	0.738	0.950	0.797	8.74	0.084	0.109	5.2
2	111	5.594	5.153	0.645	0.204	20.29	0.254	0.032	15.1
3	111	3.406	3.143	0.273	0.010	12.52	0.251	0.021	21.4
3	10	0.387	0.337	0.180	0.130	1.78	0.189	0.101	4.0
4	111	2.323	1.537	0.811	0.025	12.10	0.127	0.067	43.9
4	60	2.873	2.512	0.593	0.232	8.85	0.284	0.067	19.0
4	10	0.520	0.371	0.283	0.135	1.76	0.211	0.161	7.8

Each bladder was studied at least twice. The studies are presented in the order in which they were performed. Rate coefficients and fluxes are expressed as in Table I. The pool is given in μEq·100 mg dry weight^{-1}. SCC = short-circuit current, and is expressed in μamp·cm^{-2}.

a characteristic of a saturating system. On the other hand, as already mentioned, vasopressin induces a rise in both the rate coefficient for the pump and the pool size, clearly indicating that the rise in the former cannot be secondary to a rise in the latter.

In order to demonstrate this even more clearly, the studies shown in Table III were performed. Each of these bladders was studied at a decreased sodium concentration in the absence and in the presence of vasopressin. In addition, two of them were studied in the presence of normal Ringer solution as well as at low sodium concentrations. In this case, values for the pool size were calculated on the assumption of the steady state, even after the addition of

vasopressin. There are two reasons for this. In the first place, there is clearly a difference in the ADH response of the toad bladder in the presence of a normal Ringer solution, as compared to that in the presence of a low sodium concentration. The main difference is that the response is more prolonged, and decays far more slowly. This is indicated by Fig. 2, which shows the response of the same bladder treated with vasopressin in normal Ringer solution and in 30 mM sodium. Thus, the assumption of a steady state appears to be more valid in the low sodium situation. Second, for the purpose of the comparison being made, it is necessary to calculate a pool size. It should be clear that in the absence of a steady state, in which mucosal entry of sodium is increased far more than is serosal exit, the calculation of pool size will result in an underestimate of the real value. Hence, the conclusions to be drawn from the present argument will be understated, rather than over-stated, by the steady-state assumption. As seen from Table III, the effect of vasopressin is, as shown before in normal Ringer, to increase both the pump rate constant and the pool size. These points are brought out better in Fig. 3, in which the pools are plotted against the rate coefficients. Values for a given bladder are connected. The dotted lines connect the vasopressin values, shown as open circles, with their controls studied at the same external sodium concentration. It can be seen that the only times in which a positive slope obtains are in the vasopressin studies. Hence, it is clear that one can dissociate the effect of vasopressin on the pump from its effect on the pool size.

It is clear from the studies reported here that there is a single measurable transport pool in the toad bladder. As discussed previously, J_{s1}, the pump flux, is calculated as the product of the pool and the pump rate coefficient,

TABLE III
EFFECTS OF ADH WTIH
LOW AMBIENT SODIUM

	J_{1M}	J_{M1}	J_{S1}	J_{1S}	Pool	k_{M1}	k_{S1}	SCC
111 mM	1.835	1.422	0.492	0.079	6.92	0.206	0.071	36.4
20 mM	0.300	0.225	0.149	0.074	1.48	0.153	0.101	13.4
20 mM + ADH	0.295	0.120	0.327	0.152	1.74	0.069	0.188	31.4
30 mM	0.184	0.093	0.252	0.162	1.30	0.071	0.194	6.2
30 mM + ADH	1.252	0.479	1.639	0.865	7.05	0.068	0.233	53.3
50 mM	0.811	0.747	0.213	0.149	4.95	0.151	0.043	4.2
50 mM + ADH	1.107	0.657	0.543	0.094	7.36	0.089	0.074	29.3
111 mM	3.453	3.293	0.219	0.059	7.29	0.452	0.030	10.9
60 mM	0.815	0.366	0.389	0.060	2.28	0.160	0.171	26.1
60 mM + ADH	1.175	0.613	0.731	0.169	3.78	0.162	0.194	32.7

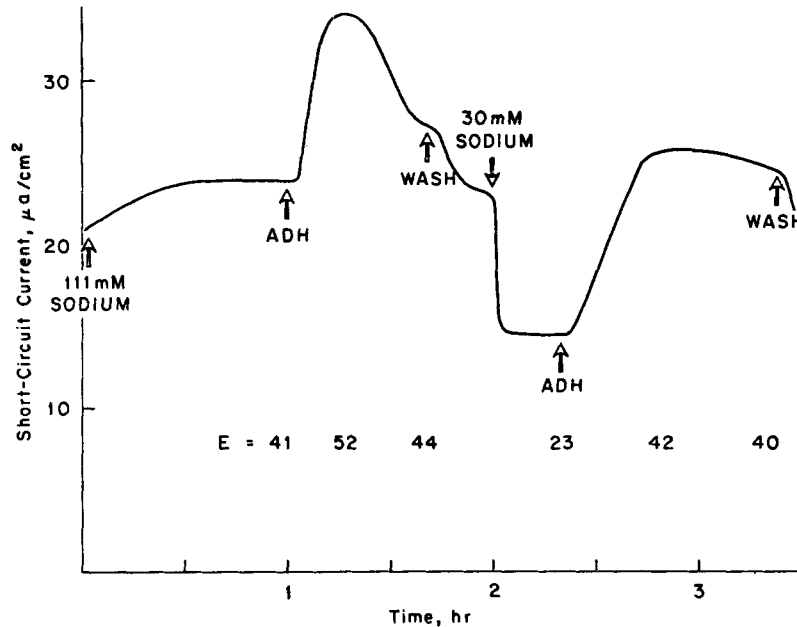Data are presented as in Table II.

FIGURE 2.   Effect of ADH at different sodium concentrations. The bladder was mounted in normal Ringer (111 mM Na), and allowed to reach a stable short-circuit current. Where indicated, vasopressin was added to the serosal medium, and subsequently washed off with normal Ringer free of hormone. Solutions containing 30 mM Na (choline replaced the rest of the sodium) were then placed in both chambers, and ADH was again added to the serosal medium. The gradual onset of the response, and the far slower decay, are obvious. E = transepithelial potential difference at the times shown.

k_{S1}. On the other hand, this gives no evidence as to whether or not all the sodium that traverses the "pump" does so by a single mechanism. In particular, we have provided no evidence as to whether or not there is a component of exchange diffusion between the transport compartment and the serosal compartment. If such were present, it may be that the action of vasopressin is to accelerate such exchange, rather than to directly increase the unidirectional pump rate. As seen from Table III, there is a rise in J_{1s} in three of the four studies at low sodium concentration, indicating at least the possibility of an increase in an exchange component. This calculation is complicated by the fact that it requires a steady state, and that very small errors in the calculated pool size (as a result of the nonsteady state) will result in large changes in the unidirectional serosal flux rates, when compared to the steady-state control values. Nonetheless, these changes are not statistically significant, and if one calculates the transepithelial serosa-to-mucosa flux, J_{MS} (from the fact that $J_{MS} = J_{M1}J_{1S}/J_{M1} + J_{S1}$, which may be derived by reasoning similar to that employed to derive equation (26) in the accom-
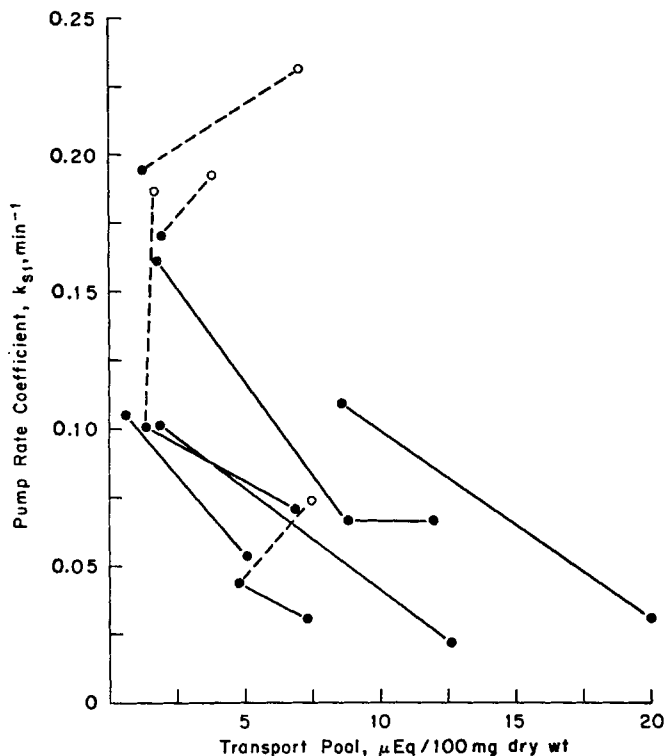
FIGURE 3.   Relationship between k_{S1}, the pump rate coefficient, and A_1, the size of the transport pool. Each bladder was studied at least twice. Lines connect points from the same bladder. The dotted lines connect experiments carried out in the absence (solid circles) and in the presence (open circles) of vasopressin. The data are those shown in Tables II and III. Note that whereas an increase in sodium concentration in the media (and in the transport pool) effects a decrease in the pump rate coefficient, the addition of vasopressin results in an increase in both the pool size and the rate coefficient.

panying paper (Finn and Rockoff, 1971; Ussing and Zerahn [1951]) from the data, there was an increase in two and a decrease in two of the studies following vasopressin; again, these differences are not significant. Data published elsewhere also indicate that vasopressin does not affect the transmural serosa-to-mucosa flux (Leaf and Dempsey, 1960). Still, the possibility exists that a part of the vasopressin-stimulated efflux into the serosal solution is accomplished by the mechanism of exchange for sodium in the serosal compartment, so that the increase in the rate constant observed above may represent some action of vasopressin on an exchange component.

In order to evaluate this possibility, studies were performed in which all the sodium on the serosal side was replaced with choline. Under such circumstances, there is a tendency for the short-circuit current to decline slowly, although this is not always the case. In fact, in most experiments performed

here, the short-circuit current was as stable in the presence of serosal choline as in its absence.[1]

Washout experiments were done before and after the addition of vasopressin to the serosal medium, as before. It was of interest that the response to the hormone seems to be quite different in the absence of serosal sodium than in its presence. Fig. 4 shows a typical experimental result; vasopressin was first added to a bladder bathed in normal Ringer, and subsequently the hormone was added in the absence of serosal sodium. It is to be noted that the
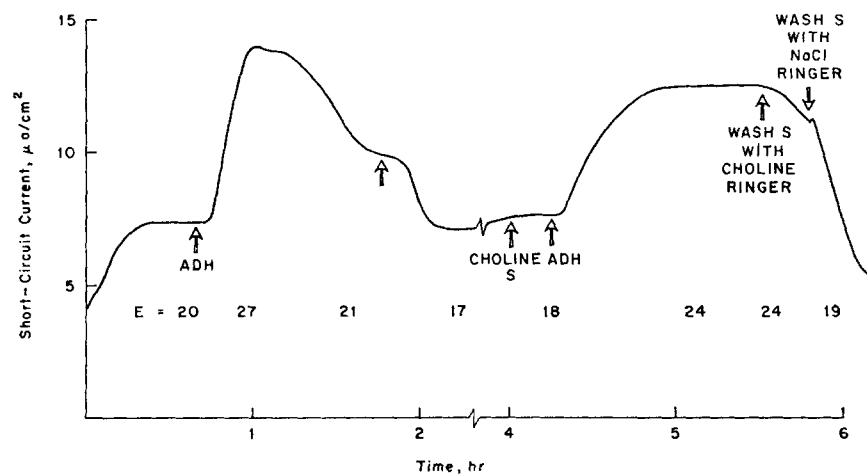

FIGURE 4. Effect of ADH in the absence of serosal sodium. The first portion of the experiment is as in Fig. 2. At the second arrow, the hormone is washed off with sodium Ringer. All sodium in the serosal bath was replaced with choline where indicated, and vasopressin was again added. The slow onset and constant maximal value are shown. It is also apparent that the hormonal effect is washed off more slowly with choline Ringer than with sodium Ringer.

onset of the hormone effect in the latter case is delayed, that the rise to a peak is also prolonged, and that the maximum response is maintained, often for as long as 2 hr. This result is similar to that noted above for media with equal, but diminished, sodium concentrations in both media.

Thus in the washout experiments, studies could be performed long after the addition of hormone, at a time when the short-circuit current was quite stable, and when the steady-state assumption could reasonably be made. The results, shown in Table IV, indicate that there is an increase in k_{s1} after ADH, and that the magnitude of the increase is similar to that seen when sodium is present in both media. Thus, the effect of vasopressin on the sodium

---

[1] It has been reported that the removal of sodium from the serosal medium results in a dramatic decrease in the short-circuit current and in the mucosa-to-serosa flux (Leaf, 1966); such an effect was never seen here, nor could it be demonstrated by Herrera (1968).

flux at the serosal side of the transport pool cannot be ascribed to an effect on sodium-sodium exchange.[2]

As previously noted, there is another sodium compartment in the toad bladder; evidence was presented that this pool is unlikely to be involved sig-

TABLE IV

EFFECTS OF ADH IN THE
ABSENCE OF SEROSAL SODIUM

	k_{M_1}		k_{S_1}		SCC	
	Control	ADH	Control	ADH	Control	ADH
	0.274	0.351	0.008	0.033	10.2	33.1
	0.390	0.251	0.029	0.058	6.1	12.6
	0.186	0.180	0.077	0.122	9.2	19.0

Data are expressed as in Table II. All bladders were mounted in normal Ringer and allowed to stabilize. The serosal solution was replaced with sodium-free Ringer, and a washout was performed. Subsequently, vasopressin was added, and the washout repeated 45–60 min later.

TABLE V

EFFECTS OF ADH ON THE
SLOW TISSUE POOL

	k_{M_2}		k_{S_2}		J_{2M}		$P_2(0)/P_M(0) \times 100$	
	Control	ADH	Control	ADH	Control	ADH	Control	ADH
1	0.039	0.045	0.0102	0.0115	0.453	0.692	0.526	0.702
2	0.024	0.021	0.0040	0.0040	0.270	0.370	0.584	0.842
3	0.055	0.050	0.0012	0.0008	0.322	0.285	0.340	0.335
4	0.056	0.055	0.0012	0.0017	1.182	1.130	0.681	0.551
5	0.088	0.014	0.0033	0.0069	1.438	1.416	1.249	1.056
6	0.004	0.023	0.0001	0.0004	0.175	0.447	1.623	1.168
7	0.033	0.062	0.0032	0.0020	0.585	0.883	0.839	1.017
Mean	0.043	0.039	0.0033	0.0039	0.632	0.746	0.835	0.814
± SEM	± 0.010	± 0.006	± 0.0013	± 0.0015	± 0.184	± 0.159	± 0.170	± 0.113
Δ	−0.004		0.0006		0.114		−0.024	
± SEM	± 0.013		± 0.0006		± 0.058		± 0.096	
p	N.S.		N.S.		N.S.		N.S.	

Data are expressed as in Table I.

[2] It is possible, of course, that despite the small diffusion pathway from the transport pool to the serosal bath, and despite the fact that the bladder is exposed to constantly flowing sodium-free medium for at least an hour before the tracer is added and for another 45–60 min afterward, that there is some sodium present for exchange at the transport site. It is likely that the concentration would be quite low, however, so that any postulated vasopressin-stimulated exchange component would have to have extremely high affinity for sodium.

nificantly in transepithelial transport. Briefly, that evidence was: (a) trans-epithelial influxes calculated from the unidirectional flux rates in this pool were far smaller than the directly measured transepithelial influxes, (b) a pool with similar characteristics was present after the center portion of the bladder was removed, and (c) there was no effect on this pool when cyanide or a large bucking potential was applied. In the present studies, more rigorous evidence may be marshalled. As shown in Table V, the size and flux rates for this pool may be calculated before and after the addition of vasopressin. As is clearly seen, there was no effect of the hormone on any of these parameters.

DISCUSSION

The findings in the present paper confirm and extend those reported previously (Finn, 1969; Finn and Rockoff, 1971). The major observation concerns the effects of vasopressin on the transport of sodium across the toad bladder.

It has been known for some time that this hormone exerts a stimulatory effect on sodium transport in both frog skin (Ussing and Zerahn, 1951) and toad bladder (Leaf et al., 1958), as well as in other epithelial structures. In addition, the hormone increases the passive permeability of these tissues to water (Koefoed-Johnsen and Ussing, 1953; Hays and Leaf, 1962). In the latter case, it has been shown clearly that the site of action of the hormone is on the mucosal side of the cells (MacRobbie and Ussing, 1961; Peachey and Rasmussen, 1961), although it must be added to the serosal medium to have any effect. Studies of the site of action of the hormone on sodium transport, on the other hand, have not been so definitive. It has been thought that the hormone affects the mucosal barrier to sodium (Frazier et al., 1962; Frazier and Hammer, 1963; Civan and Frazier, 1968), and the present work offers direct confirmation of this. As shown in Table I, there is, on the average, a 77% increase in the influx of sodium from the mucosal chamber into the transport pool. The electrochemical potential gradient for sodium at this border is not known, but it is highly likely that under the conditions of these experiments the passage of sodium into the cells from the mucosal medium is not an energy-dependent process. Frazier (1962) has shown that there is a small electronegative well within the cells in the short-circuited toad bladder. Although the electrical potential profile is not known during short circuit following the addition of vasopressin, Civan and Frazier (1968) have shown that under open-circuit conditions there is little or no change in the potential across the mucosal barrier following the addition of the hormone. We can therefore postulate that however vasopressin acts to enhance entry of sodium, it does so without initially altering the electrochemical potential gradient for this ion. On the other hand, it cannot be stated that vasopressin simply

increases the sodium permeability of the mucosal facing barrier, since there was no change in k_{M1}, the rate coefficient for exit of sodium from the cells to the mucosal medium. The studies reported here also indicate that vasopressin addition results in a large increase in the rate coefficient for exit of sodium at the serosal side, and that this effect does not require the presence of sodium in the serosal medium. Furthermore, although there is an increase in the size of the transport pool, this increase does not of itself explain the rise in the pump rate constant, since, as shown in Fig. 3, the rate constant ordinarily declines with increases in the pool.

On the other hand, the increase in the pool size, presumably reflecting an increase in sodium concentration in the pool, would tend to increase any component of passive sodium movement from the cells to the serosal medium. However, it seems clear that there is an electrochemical potential gradient in both the control (Frazier, 1962) and vasopressin-treated situation (Civan and Frazier, 1968) which opposes such movement. Therefore, any effect of the hormone which would increase the passive permeability properties of the serosal barrier would necessarily result in an increase in the net movement of sodium from the serosal medium into the cells, quite the opposite from the situation which actually exists. It seems quite likely, therefore, that the vasopressin effect is, in fact, on the pump mechanism itself.

One must raise the question, however, as to whether or not the hormone might be affecting, in addition, a passive property on the serosal side of the transport compartment. As previously mentioned, in some of the experiments, there was an effect of vasopressin on the calculated backflux, J_{1S}. Although the latter effect was not so consistent as the effect on the pump rate coefficient, the possibility exists that, since there is an effect of the hormone on both fluxes at that border, there might be an effect on the passive properties of this barrier. There is no good way to exclude this possibility completely, but the present evidence (namely, that (a) as stated above, there must be an effect on the pump since an effect on a passive process alone would result in a decrease in the net cell-to-serosa flux, and (b) the effect of the hormone on k_{S1} is the same whether or not sodium is present in the serosal medium) is strongly suggestive of a single effect of the hormone on the pump pathway.

It is of interest that Frazier and Hammer (1963) performed sodium washout experiments by a technique similar to that used here, and concluded that vasopressin had an effect on the mucosal border. However, in their studies, no data points were obtained until at least 7 min of washout of the chamber (and bladder) had occurred, so that this technique would have missed any component with a half-time as fast as that described here for the transport pool. Furthermore, it is difficult to evaluate their data since their effluent curves are clearly multiexponential (as shown here) but do not appear to be

parallel. In order to evaluate the pools and kinetics, it is necessary that the curves be fit to sums of exponentials in which the exponential terms are equal for the two curves (Finn and Rockoff, 1971); hence it is difficult to evaluate those experiments quantitatively.

The decrease in rate coefficient with the increasing pool size would be expected for a saturating system, in which the flux rate, J_{ij}, would increase to a limit as substrate concentration, A_j (the sodium transport pool), rises. Presumably, over some finite range, the flux would increase linearly; over that range, the rate coefficient for efflux, k_{S1}, would remain constant, from the equation $J_{S1} = P_1 k_{S1}$. However, as soon as the flux rate departs from linearity, the rate coefficient would become a decreasing function of the pool size, so that at full saturation (J_{S1} = constant) a plot such as that shown in Fig. 3 would yield a rectangular hyperbola. In order to define the behavior of the system more fully, it will be necessary to examine this relationship over a fairly wide range of substrate concentrations. Nonetheless, it is clear from the data presented that the sodium pump is saturating over the level of concentrations normally examined, since the pump rate coefficient is a decreasing function of the pool.

This relationship has also been tested at higher substrate levels. One can increase mucosal entry of sodium with the drug amphotericin B (Finn, 1968, 1970). Under such circumstances, washout data indicate that there is a rise in J_{1M}, J_{M1}, and A_1, as with vasopressin, but a fall in k_{S1}.[3] The latter would, of course, be predicted from the data in Fig. 3. Thus, the action of this drug does not entirely mimic the action of vasopressin, confirming the previous conclusions from this laboratory (Finn, 1968).

It is unfortunate that conditions make it difficult to quantify the size of the sodium pool after the additon of vasopressin, owing to the transient nature of the response in normal Ringer. On the other hand, the action of the hormone seems to be prolonged when the sodium concentration is lowered, so that valid determinations of the sodium pool can be made. The results, as seen in Tables III and IV, and Fig. 3, again underline the distinct duality of the sites of action of the hormone.

As shown here, however, the most prolonged effect of vasopressin is seen when choline replaces all sodium in the serosal solution. The response begins at about the same time, rises to a peak much more slowly, and is often maintained for 2 hr or more. Furthermore, the hormone effect (and possibly the hormone itself) is washed off more slowly. The reasons for this are not clear, but the phenomenon is currently under investigation. Again, the effect on the pump rate coefficient is clear, indicating that the vasopressin effect on the pump does not require the presence of sodium in the serosal medium.

With regard to changes in the sodium concentration in both media, it is of

[3] A. L. Finn. Unpublished observations.

considerable interest that the present model yields consistent, reasonable values under such conditions. Thus, there is a decrease in the pool size and an increase in k_{s1} when the sodium concentration is lowered. In the present studies, the effects of changes in sodium concentration on the fluxes at the mucosal side have not been discussed. This is a subject of considerable interest, and requires, like evaluation of the nature of the saturation process, detailed analysis over the entire range of ambient sodium concentrations. At the present time it is clear that both mucosal and serosal fluxes are affected by changes in the sodium concentration in the media. The results of these studies will be the subject of a future communication. It should be added, however, that the effects of changes in the sodium concentration are also restricted to the fluxes and rate constants of the faster of the two tissue compartments, as are the effects of vasopressin.

Thus, the data presented indicate (a) that the technique of compartmental analysis may be applied to the toad bladder sodium transport system, (b) that the transport pool for sodium, and its kinetics, may, in fact, be determined, (c) that vasopressin has two separate sites of action: first, it increases the entry of sodium into the transport pool, and thereby effects a rise in the magnitude of that pool, and second, it has a direct stimulatory effect on the serosal sodium pump (presumably both actions are mediated by cyclic AMP (Orloff and Handler [1962]) and (d) that the sodium transport system behaves as a saturating system.

BIBLIOGRAPHY

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

CIVAN, M. M., O. KEDEM, and A. LEAF. 1966. Effect of vasopressin on toad bladder under conditions of zero net sodium transport. *Amer. J. Physiol.* 211:569.

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. 1969. Kinetics of sodium transport in the toad bladder. Proceedings 3rd International Biophysical Congress. Cambridge, Massachusetts. p. 250.

FINN, A. L. 1970. Effects of potassium and amphotericin B on ion transport in the toad bladder. *Amer. J. Physiol.* 218:463.

FINN, A. L., and M. L. ROCKOFF. 1971. 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.

FRAZIER, H. S., E. F. DEMPSEY, and A. LEAF. 1962. Movement of sodium across the mucosal surface of the isolated toad bladder and its modification by vasopression. *J. Gen. Physiol.* 45:529.

FRAZIER, H. S., and E. I. HAMMER. 1963. Efflux of sodium from isolated toad bladder. *Amer. J. Physiol.* 205:718.

HAYS, R. M., and A. LEAF. 1962. Studies on the movement of water through the isolated toad bladder and its modification by vasopressin. *J. Gen. Physiol.* **45**:905.

HERRERA, F. C. 1968. Action of ouabain on bioelectric properties and ion content in toad urinary bladder. *Amer. J. Physiol.* **215**:183.

KOEFOED-JOHNSEN, V., and H. H. USSING. 1953. The contributions of diffusion and flow to the passage of D_2O through living membranes. *Acta Physiol. Scand.* **28**:60.

LEAF, A. 1966. On the functional structure of the transport system in the toad bladder. Proceedings 3rd International Congress of Nephrology. Washington, D.C. **1**:18.

LEAF, A., J. ANDERSON, and L. B. PAGE. 1958. Active sodium transport by the isolated toad bladder. *J. Gen. Physiol.* **41**:657.

LEAF, A., and E. F. DEMPSEY. 1960. Some effects of mammalian neurohypophyseal hormones on metabolism and active transport of sodium by the isolated toad bladder. *J. Biol. Chem.* **235**:2160.

LIPTON, P., and I. S. EDELMAN. 1969. Effects of regulatory hormones on intracellular Na^+ and K^+ of toad bladder epithelial cells. Abstracts of the Biophysical Society 13th Annual Meetings. Los Angeles, California. 164.

MACROBBIE, E. A. C., and H. H. USSING. 1961. Osmotic behavior of the epithelial cells of frog skin. *Acta Physiol. Scand.* **53**:348.

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

ORLOFF, J., and J. S. HANDLER. 1962. The similarity of effects of vasopressin, adenosine-3',5'-phosphate (cyclic AMP) and theophylline on the toad bladder. *J. Clin. Invest.* **41**:702.

PEACHEY, L. D., and H. RASMUSSEN. 1961. Structure of the toad's urinary bladder as related to its physiology. *J. Biophys. Biochem. Cytol.* **10**:529.

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