ACTIVE SODIUM TRANSPORT BY THE ISOLATED TOAD BLADDER*· ‡

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ABSTRACT

Studies were made of the active ion transport by the isolated urinary bladder of the European toad, *Bufo bufo*, and the large American toad, *Bufo marinus*. The urinary bladder of the toad is a thin membrane consisting of a single layer of mucosal cells supported on a small amount of connective tissue.

The bladder exhibits a characteristic transmembrane potential with the serosal surface electrically positive to the mucosal surface. Active sodium transport was demonstrated by the isolated bladder under both aerobic and anaerobic conditions. Aerobically the mean net sodium flux across the bladder wall measured with radioactive isotopes, Na^{24} and Na^{22}, just equalled the simultaneous short-circuit current in 42 periods each of 1 hour's duration. The electrical phenomenon exhibited by the isolated membrane was thus quantitatively accounted for solely by active transport of sodium. Anaerobically the mean net sodium flux was found to be slightly less than the short-circuit current in 21 periods of observation. The cause of this discrepancy is not known.

The short-circuit current of the isolated toad bladder was regularly stimulated with pure oxytocin and vasopressin when applied to the serosal surface under aerobic and anaerobic conditions. Adrenaline failed to stimulate the short-circuit current of the toad bladder.

INTRODUCTION

Although the ability to perform active transport of ions is common to living cells in general, considerable technical difficulties are encountered in the study of this process in most tissues. Much useful information has been obtained from the study of homogeneous cell types such as red blood cells and yeast. Much has been added to knowledge of ion transport, particularly in nerves, by study

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of individual cells. However, facts have perhaps accumulated most rapidly recently from a consideration of membranous tissues specialized in the active transport of one or more ions. Frog skin, gastric mucosa, intestinal epithelium, and gills are examples of such specialized membranes. The study of sodium transport by the frog skin has progressed rapidly through the work of Ussing and associates especially since their introduction of the short-circuit current technique (1). The complexity of the frog skin with its stratified epithelial layers, skin glands, and thick subcutaneous connective tissue layer makes it unsuitable, however, for studies in which the correlation of the active ion transport process to biochemical events in those cells engaged in this process is sought. A membrane of simpler histological structure in which the cells performing the active ion transport comprise a larger fraction of the total cells of the tissue would be desirable. This paper is concerned with the general description of the active sodium transport process in the isolated toad bladder, a membrane which at least partially fulfills this requirement for simplicity of structure.

Methods

Studies were made on the European toad, *Bufo bufo*, and on the larger American species, *Bufo marinus*. The former were stored in a cool cellar and used unfed during the fall season. The latter were used throughout the year, kept at room temperature, and force fed with meal worms once or twice weekly. The animals were pithed, and one-half of the bladder removed, rinsed in frog Ringer (Na, 113.5; K, 1.88; HCO_3, 2.38 m. eq./liter and Ca, 0.89 mM/liter) and mounted between two halves of a lucite chamber similar to that described by Ussing and Zerahn (1). The cross sectional area of the chamber was 3.14 cm.^{2} and 10.0 ml. of Ringer was usually used to bathe each side of the membrane. Short-circuit current measurements were made according to the technique of Ussing and Zerahn (1).

The net sodium flux was determined simultaneously with the short-circuit current measurements using radioactive Na^{22} to measure influx (mucosal to serosal side) and Na^{24} to measure simultaneously the outflux (serosal to mucosal side). The double isotope labelling technique described by Levi and Ussing (2) was used. Na^{24} was obtained from Oak Ridge and Na^{22} from Nuclear Science and Engineering Corporation, Pittsburgh. All counts were made to $\pm$ 1 per cent standard deviation. Appropriate corrections were applied for the decay of the Na^{24}. All Na^{22} counts were made after 3 weeks had elapsed to allow for complete decay of the Na^{24}. The Na^{24} count were made in the presence of Na^{22} by using an aluminum filter (69.12 mg./cm.^{2}) to absorb the majority of the softer radiations from Na^{22}. Na^{24} counting with filters was done with a tracerlab 100 scaler and an end window G-M tube with manual sample changer. The Na^{22} was counted with a model D47 gas flow counter with a micromil window and a nuclear-Chicago scaler with automatic sample changer.

Short-circuit current measurements were made with a Weston D.C. micro-ammeter, model 622, having a full scale reading at full sensitivity of 50 microamperes and a precision of 0.5 per cent full scale reading. Membrane potentials were measured with a model 3 radiometer pH meter.

The neurohypophyseal hormone preparations were pitressin (Parke, Davis and Co.) and pure oxytocin, ON5, and vasopressin, AVN3, (the two pure hormone preparations were generously supplied by Professor V. du Vigneaud). The hormone was added directly to the fluid bathing the serosal or mucosal surfaces of the membrane. Adrenaline hydrochloride (Parke, Davis and Co.) was used in some experiments.

Total solute concentrations of serum and urine were determined cryoscopically using a Fiske osmometer. Sodium and potassium concentrations were measured on a Baird flame photometer using a lithium internal standard. Chloride was measured potentiometrically by the method of Sanderson (3) with 0.2 N nitric acid replacing the acetic acid to reduce potential drift. Wet tissue weights were obtained after blotting the bladder carefully on Whatman No. 54 filter paper and weighing in tared vessels. Dry weights were subsequently obtained by drying overnight in an oven at 105°C.

To obtain anaerobic conditions the medium was equilibrated with high purity N_2 (Linde Co., Needham Heights, Massachusetts) after first bubbling the tank gas through a double train of vanadyl sulfate solution as recommended for deoxygenation by Meites and Meites (4). A vibrating platinum microelectrode (5) was used for oxygen measurements to determine the time required to obtain anaerobic conditions in the chamber when nitrogen replaced air as the gassing phase.

RESULTS

Structure.—The urinary bladder of the toad is a bilobed highly distensible organ which in the hydrated animal may occupy one-third to one-half of the entire abdominal cavity. It is transparently thin and consists of a single layer of epithelial cells lining the mucosal surface. These cells are supported on a fine layer of connective tissue. Small bundles of smooth muscle are scattered through this layer as is the blood supply to the bladder. The smooth muscle bundles are dispersed and do not form a continuous sheath. They appear to function only to keep the size of the bladder proportional to its fluid content; actual expulsion of urine is accomplished by the heavy musculature of the abdominal wall. As most of the bladder lies free in the peritoneal cavity much of its surface is covered by a layer of serosal cells.

Fig. 1 shows histological sections of this delicate membrane stained with hematoxylin and eosin. Cross sections of 125- and 450-fold magnification are shown. As the sections were prepared by tying off one-half bladder, excising it proximal to the ligature, and dropping the resulting urine-filled balloon directly into 10 per cent formalin fixative the thinness of the tissue represents its physiological state. A greater or lesser urine content would have modified the histological picture by further stretching or contracting the membrane. In the empty contracted state more smooth muscle and a thicker submucosal connective tissue layer are apparent. The higher magnification of the membrane shows the relative thickness of mucosal cells to total membrane thickness in a physiological state of distention.

Composition of Urine and Serum.—Table I shows the total solute, sodium,

potassium, and chloride concentration of toad's serum and urine. The serum concentrations are readily altered by the state of hydration of the animal. Values are arranged in order of increasing overhydration with the most hypotonic serums at the bottom of the table. The concentrations in the correspond-

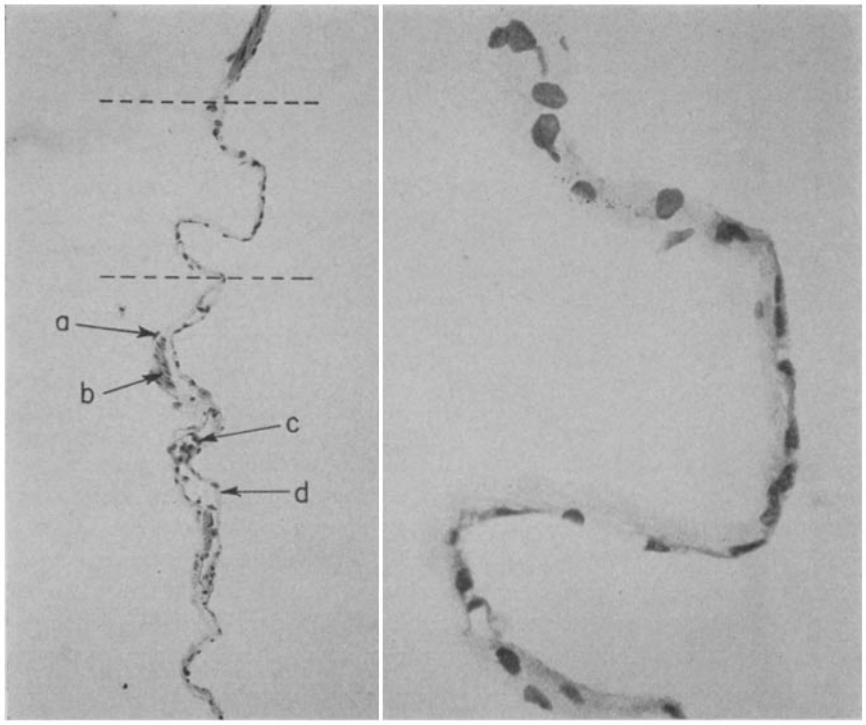

FIG. 1. Histologic sections of cross section of toad bladder. Tissue was fixed in 10 per cent formalin and stained with hematoxylin and eosin. On the left magnification is 125-fold, and arrows show (*a*) serosal lining, (*b*) bundle of smooth muscle cells, (*c*) small blood vessel containing nucleated red blood cells, (*d*) mucosal epithelium. On the right is shown a higher magnification (× 450) of the length of bladder mucosa indicated in the low power section between the two broken lines to show the relative thickness of mucosal epithelial cells to supporting tissue in the bladder wall.

ing bladder urine are shown to the right. In all instances urine sodium and chloride concentrations are considerably lower than the corresponding serum values. Some values of urine sodium concentration as low as that achieved by the mammalian renal tubule indicate a powerful reabsorptive capacity for this ion. In accordance with recent results reported by Sawyer (6) values for urins concentration hypertonic to serum have not been found. Urines as dilutee a the human kidney can elaborate were found in the overhydrated toads.

Active Sodium Transport.—When bathed in a sodium-containing Ringer

solution and with the same concentration of sodium on both sides of the membrane the serosal side of the bladder was invariably found to be electrically positive relative to the mucosal surface. Spontaneous membrane potentials

TABLE I

Sodium, Chloride, Potassium, and Total Solute Concentrations in Toad's Serum and Urine

Experiment	Total solute concentration	Serum			Total solute concentration	Urine		
		Na	K	Cl		Na	K	Cl
	m.osM/liter	*m.eq./liter*			*m.osM/liter*	*m.eq/liter*		
1	297	134	3.7	98	287	4.3	15	5.8
2		129	4.0	96	302	4.5	10.7	18.4
3*	230	109	—	75	103	3.0	1.5	—
					116	26.0	3.0	21
					136	27.3	3.0	28
					71	7.4	2.8	9.9
					82	19.2	2.9	7.6
					119	29.6	3.1	20.6
4	224	109	3.1	80	126	2.8	15.1	8.8
5*	218	109	3.2	80	161	19.0	12.3	18.2
					47	9.5	1.5	6.3
					50	1.8	1.3	3.3
					96	0.5	3.0	11.2
					74	4.8	1.0	12.9
6	204	96	4.0	69	148	5.0	10.2	18.8

* Pooled serum used for these analyses.

TABLE II

Comparison of Net Sodium Flux and Short-Circuit Current through Isolated Toad Bladder; the Aerobic Active Sodium Transport

Toad	No. of periods	Duration each period	Mean Na influx	Mean Na outflux	*A* Mean net Na transport	*B* Mean short-circuit current
		min.	*μ eq./cm.²/min.*			
B. bufo	26	60	0.0206	0.0121	0.0085	0.0085
B. marinus	16	60	0.0357	0.0095	0.0262	0.0268

	B. bufo	*B. marinus*
Mean difference (*A-B*)	0	−0.00065 μ eq./cm.²/min.
S.E. of mean difference	0.00031 μ eq./cm.²/min.	+0.0018
Mean dry weight	1.3 mg./cm.²	3.43 mg./cm.²

of 120 mv. have been measured but usually values of 20 to 50 mv. were obtained with *Bufo marinus* and values of 5 to 20 with *Bufo bufo*. Ussing and Zerahn (1) have shown that, with the same medium on either side of the isolated frog skin, when an external potential is applied across the skin so as to reduce

the spontaneous membrane potential to zero, the current measured in the external circuit just equals the active sodium transport through the skin. Table II shows the mean results of simultaneous measurements of sodium fluxes from mucosal to serosal surfaces (influx) with Na^{22} and from serosal to mucosal surfaces (outflux) with Na^{24} and the short-circuit current in 26 periods

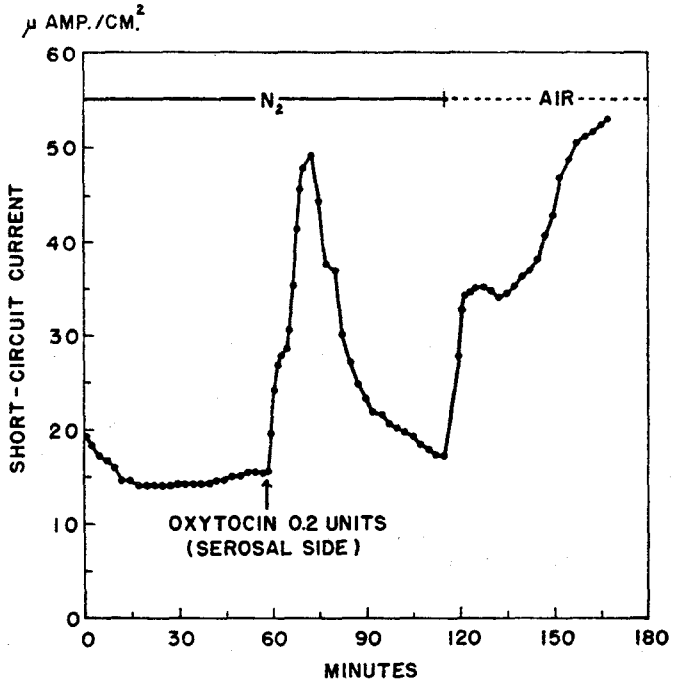

FIG. 2. Anaerobic active sodium transport by isolated toad bladder and its stimulation by neurohypophyseal hormone. Pure nitrogen has been substituted for air 20 minutes before short-circuit current measurements were commenced. Active sodium transport is plotted on the ordinate and expressed as short-circuit current in μ amp./cm.^{2} while time in minutes is shown on the abscissa. The neurohypophyseal hormone was pure oxytocin and the amount of hormone solution added was 25 μl.

of 1 hour each on the European toad, *Bufo bufo*, and 16 periods on the larger American toad, *Bufo marinus*. When expressed in the same units excellent agreement is found between these two independent measurements in both species of toad. As has been demonstrated for the frog skin (1, 7–10) these results constitute the proof that sodium is actively transported by this membrane and that it accounts quantitatively for the total electrical activity of the short-circuited bladder. Although the active ion transport expressed per unit area of membrane is less for *B. bufo* than for *B. marinus*, when corrected to

unit dry weight the rates of transport are not dissimilar—0.0065 and 0.0077 μ eq./mg. dry weight/minute, respectively. The larger bladder of *B. marinus* could be mounted more easily leaving more tissue in the chamber.

It has been shown for the frog skin (5) that some short-circuit current per-

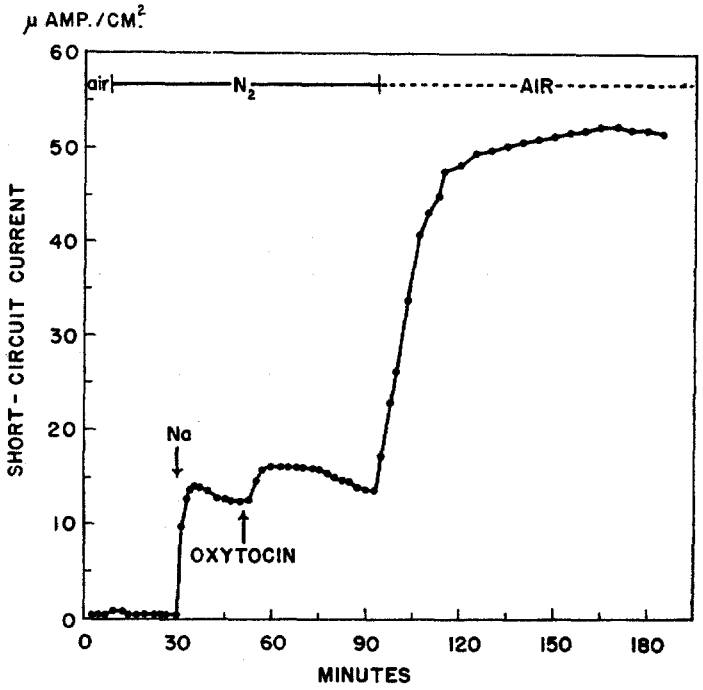

FIG. 3. Initiation of sodium transport by the isolated toad bladder under anaerobic conditions. The bladder was mounted in a sodium-free magnesium Ringer in which no sodium transport was demonstrable initially either aerobically or anaerobically. After 30 minutes anaerobically deoxygenated sodium Ringer was added and sodium transport commenced. This sodium transport was increased slightly by 0.2 unit of pure oxytocin and greatly by air.

sists for periods of an hour or two under completely anaerobic conditions and even in an atmosphere of pure carbon monoxide. Fig. 2 shows short-circuit current measurements obtained from an isolated toad bladder which was maintained over 2 hours under completely anaerobic conditions. Pure nitrogen had been substituted for air 20 minutes before the short-circuit measurements were commenced. The time required to render the Ringer solution oxygen-free by gassing with nitrogen was determined directly on the chamber used in this study with a vibrating platinum microelectrode (5). The mean time to reduce the oxygen concentration in the medium to < 0.08 volume per cent was 3.1

minutes and the longest time required was 5.7 minutes. In contrast to the failure of neurohypophyseal hormones to stimulate the short-circuit current of the isolated frog skin under anaerobic conditions (5), Fig. 2 shows the marked stimulation observed anaerobically for the isolated toad bladder. This was a consistent finding with both pure oxytocin (ON5) and pure vasopressin (AV-N3). When air was substituted for the nitrogen the short-circuit current rose to higher values just as had been noted previously for the frog skin.

In most instances, the short-circuit current decreases under anaerobic conditions until a new level is reached which is usually considerably lower than under aerobic conditions. This lower rate of activity anaerobically may be maintained for periods of 1 to 2 hours as shown in Fig. 2. However, in some experi-

TABLE III

Comparison of Net Sodium Flux and Short-Circuit Current through Isolated Toad Bladder; the Anaerobic Active Sodium Transport (Bufo marinus)

No. of periods	Duration each period	Mean Na influx	Mean Na outflux	*A* Mean net Na transport	*B* Mean short-circuit current
	min.		*μ eq./cm.²/min.*		
21	60	0.0174	0.0089	0.0085	0.0103
Mean difference (*A-B*)			−0.0018 μ eq./cm.²/min.		
S.E. of mean difference			±0.00059 μ eq./cm.²/min.		
∴ P = 0.01					
Mean dry weight			3.02 mg./cm.²		

ments the short-circuit current and membrane potential gradually and progressively decline to zero values. Fig. 3 shows that the short-circuit current can be generated *de novo* under anaerobic conditions. This indicates that energy supplies can be freshly mobilized under anaerobic conditions to initiate and maintain the active ion transport. When an Mg-Ringer which had all the sodium completely replaced by magnesium was used on both sides of the membrane no detectable membrane potential or shirt-circuit current could be measured. After 30 minutes of anaerobic conditions a sufficient volume of deoxygenated sodium-Ringer was added to bring the final concentration of sodium up to one-third that of ordinary frog-Ringer. Promptly on addition of sodium a short-circuit current was generated. Addition of oxytocin produced a slight further rise and addition of air a much larger rise in short-circuit current.

To determine whether the equality between short-circuit current and net sodium flux was preserved during anaerobic conditions, double isotope labelling measurements of sodium fluxes were made simultaneously with short-circuit current readings. Table III shows the mean results of such measurements in 21

anaerobic periods each of 1 hour's duration. Again the sodium influx (mucosal to serosal surfaces) was found to exceed the outflux (serosal to mucosal surfaces) but here the agreement of net flux and short-circuit current is not perfect. The short-circuit current exceeds the net sodium flux and the mean difference is found to be significant ($P < 0.01$). The cause of this discrepancy is not evident and some small systematic error in the measurements of either current or ion fluxes at these low levels of transport unfortunately cannot be excluded. It is evident, nevertheless, that active sodium transport accounts

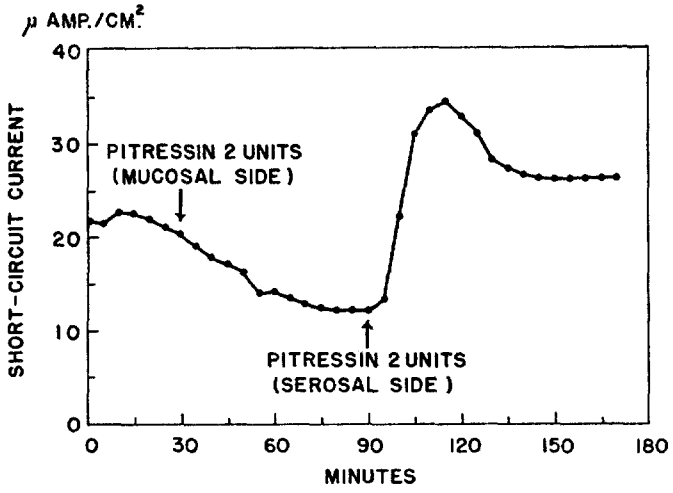

FIG. 4. Demonstration of the unilateral stimulatory effect of neurohypophyseal hormone. When neurohypophyseal hormone, pitressin, was added to the Ringer solution bathing the mucosal surface no effect on short-circuit current was noted. The same amount of hormonal preparation added to the solution bathing the serosal side resulted in a prompt and large stimulation.

for the majority of the electrical activity anaerobically even as it does aerobically. The passive flux of sodium through the membrane indicated by the mean sodium outflux values of Tables II and III during anaerobic conditions is seen to be slightly less than under corresponding conditions aerobically.

Neurohypophyseal Hormone and Adrenaline.—As in the frog skin (1) neurohypophyseal hormones produce a marked stimulation of active ion transport in the bladder. Fig. 4 shows that in this membrane also the hormone acts only unilaterally. When pitressin was added to the fluid bathing the mucosal surface it was without effect on transport. When applied to the serosal surface it resulted in a prompt large stimulation of short-circuit current. Both pure oxytocin and pure vasopressin produce this unilateral stimulatory effect which

constitutes a remarkable degree of structural specificity of the transport process considering the thinness of the membrane and the probability that only a single layer of cells is engaged in the process of sodium transport.

In contrast to its action in the frog skin adrenaline was found not to have a stimulatory effect on the toad bladder, Fig. 5. Koefoed-Johnsen, Ussing, and Zerahn (11) showed that the added current with adrenaline resulted from the active transport of chloride ions in the direction opposite to the net movement of sodium. They postulated that adrenaline stimulated secretion of the skin

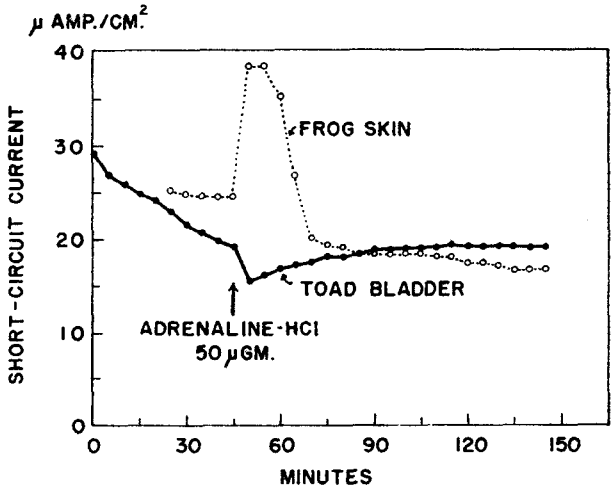

FIG. 5. Contrasting the effect of adrenaline on short-circuit current of frog skin and toad bladder. The short-circuit current of the isolated frog skin is stimulated by adrenaline. No stimulatory effect of adrenaline has been observed for the toad bladder.

glands and that this was the source for the outward movement of chloride ions. The absence of stimulation of the toad bladder with adrenaline supports this view as the toad bladder, of course, is devoid of skin glands. No explanation for the transient drop in current on addition of adrenaline is apparent.

DISCUSSION

The physiological role of the anuran bladder in maintaining water balance has been demonstrated by Steen (12), Ewer (13), and Sawyer and Schisgall (14). The present study demonstrates that sodium as well as water can be reabsorbed by the bladder mucosa. The characteristic membrane potential exhibited by the isolated bladder when both surfaces are bathed by Ringer solution of identical composition shows that the membrane is capable of net ion transport. The existence of a net sodium transport across the chemically and electrically

symmetrical short-circuited membrane is proof of an active transport mechanism for this ion species (1). In the intact animal sodium must be moved against both a chemical and an electrical gradient confirming the necessity for the performance of thermodynamic work by the bladder in the transport of sodium. Equivalence of the net sodium flux and the short-circuit current aerobically indicates that the electrical phenomenon can be entirely accounted for solely by the active transport of sodium ions. It is impossible to state at present whether the small discrepancy observed during anaerobic transport between net sodium flux and short-circuit current is physiological or constitutes a systematic error in measurement.

Ewer (13) and Sawyer and Schisgall (14) have demonstrated that injections of neurohypophyseal hormone preparations increase the rate of reabsorption of water by the anuran bladder. The present study indicates that both pure oxytocin and vasopressin stimulate transport of sodium by the bladder. This response the bladder shares with the isolated frog skin under aerobic conditions (1). The cause of the failure previously to demonstrate similar stimulation of sodium transport anaerobically by the isolated skin of *Rana temporaria* (5) is not clear. In the present study an increased short-circuit current was repeatedly demonstrated by the isolated toad bladder under anaerobic conditions with oxytocin or vasopressin.

Inasmuch as the bladder mucosa transports sodium actively it is the final determinant of the urinary sodium concentration. As bladder urine may be essentially sodium-free, see Table I, this reabsorptive mechanism for sodium must be as effective as that possessed by the renal tubules of man (15). It may be justifiably anticipated that knowledge obtained regarding the sodium reabsorptive mechanism of the toad bladder will be applicable to the problem of sodium transport by mammalian renal tubules. Because the cells transporting sodium in the toad bladder are arranged in a sheet in contrast to the myriad small tubules of the mammalian kidney, the active ion transport process is more readily studied in the anuran bladder. The fewer cell types present and the lesser amounts of supporting tissue give this membrane some advantages over the frog skin for the study of active ion transport.

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