Reversible Stimulation of Sodium Transport in the Toad Bladder by Stretch

MACKENZIE WALSER with the technical assistance of SYLVIA E. BUTLER and VALERIE HAMMOND

From the Department of Pharmacology and Experimental Therapeutics and the Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

ABSTRACT Short-circuit current and transepithelial potential difference were measured in toad hemibladders mounted as sacs on glass cannulae. When sac volume was changed by adding or removing fluid, short circuit current responded by increasing or decreasing during the ensuing half-hour. The time course of the response and its magnitude indicated that it was not artefactual. Furthermore, net sodium flux responded similarly. Sac volume, and thus bladder surface area, could be varied from 0.03 to 0.4 cm^2/mg wet weight. The mean response to either decreases or increases was 10 $\mu A/cm^2$. Everted hemibladders, however, responded less. Neither hydrostatic pressure, nor increased chloride conductance, nor increased access of oxygen or glucose to the mucosa was responsible for the response. Tissue conductance did vary markedly with volume, and may have played a role, but sodium conductance did not vary with volume in a consistent manner. The results indicate the existence of an intrinsic mechanism in this tissue which alters sodium transport in response to stretch.

INTRODUCTION

In the course of a study of the interdependence between calcium and sodium transport in the toad bladder, we observed a marked dependence of calcium transport on the degree of stretch of the tissue, expressed as area per unit of wet weight. To examine this relationship further, we mounted bladders as sacs in which stretch could be altered reversibly and reproducibly simply by adding or removing fluid. Short-circuit current (SCC) was noted to increase when volume was raised and to decrease when volume was reduced. As SCC in this tissue is a measure of the net transport of sodium (2, 3), these results pointed to the existence of an intrinsic mechanism altering sodium transport in this tissue in response to stretch. This response is the subject of the present study.

A preliminary account of this work has appeared (1).

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METHODS

Large toads (*Bufo marinus*) were kept at room temperature with access to tap water for up to 3 wk before use. They were killed by decapitation and pithing. After the abdomen was opened and the bladder dissected, a glass cannula, 1 cm in diameter, was inserted into each hemibladder and secured by a plastic ring which fitted into a groove in the cannula. Bladder tissue above the ring was cauterized and coated with a silicone lubricant. Sacs thus mounted were partially filled with normal Ringer's solution (111 mM NaCl, 3 mM $NaHCO_3$, 2.7 mM $CaCl_2$, 2 mM $MgCl_2$, 3.4 mM KCl, 5.5 mM glucose) and suspended in a beaker containing the same solution. The fluid level in the beaker was kept at or slightly above the plastic ring. Stirring and aeration in the outer solution were accomplished by a magnetic stirring bar and a bubbling device. Two different techniques were used to mix the inner solution. In the first, the cannula, 4 cm in length, remained open and stirring was achieved by a glass rod, bent at the end, inserted through the cannula into the bladder, and rotated at about 500 rpm. The inner solution was not otherwise aerated. This technique has the advantage that fluid can be quickly removed or added without pressure increments exceeding 4 cm of water; sampling is also facilitated. In the second method, a Plexiglas plug, fitted with a rubber "O" ring, was inserted into the cannula and pushed down to the bottom. Two holes in the plug admitted polyethylene tubing for inflowing and outflowing fluid. The outflow tube was connected to an open 20 ml syringe barrel several centimeters below the bladder and the inflow tube to another syringe at a comparable distance above the bladder, into which an aeration device was inserted. A pump returned fluid from the lower to the upper syringe reservoir at 6 ml/min. Changes in the volume in the bladder could be estimated from the sum of the amounts in the two syringe reservoirs. The total volume of mucosal fluid was measured at the end of the experiment. The volume in the bladder could be varied by raising or lowering both syringes. This technique made it possible to vary the volume in the bladder sac without altering the total volume or the composition of the mucosal fluid.

Electrical measurements

In both open-cannula and closed-cannula experiments, polyethylene bridges containing 1% agar in saturated KCl and tapered at the ends, entered the sac through the cannula for potential measurements and for the passage of current. The potential-measuring electrode remained at the lower end of the glass cannula within a few millimeters of the bladder. The current-passing electrode extended to approximately the center of the sac. In closed cannula experiments, the position of the electrode necessarily remained unaltered, because the polyethylene tubes fit snugly into the holes in the plug. In open cannula experiments, on the other hand, it could be moved when the bladder volume was varied so as to keep it as near the geometric center of the sac as possible (see below).

Current was passed by means of silver–silver chloride electrodes immersed in 0.1 N HCl into which the KCl-agar bridges entered. Balanced calomel electrodes served to complete the potential-measuring circuit. An automatic device for monitoring short-circuit current and potential was employed (Zubin and Walser, unpublished). The output of this instrument was recorded. The tissue was maintained short-circuited continuously except for intervals of a few seconds every 2–5 min during which open circuit potential was measured.

An element of uncertainty in the SCC measurements is introduced by the geometry of the system. Furthermore, any error attributable to this cause might well change with the volume of fluid in the mucosal sac, and thus artefactual changes in SCC with volume might occur. This problem

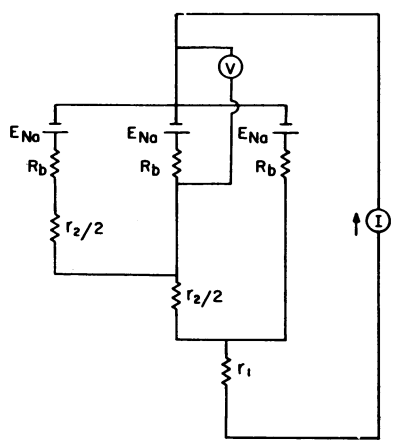

FIGURE 1 An approximate circuit to show diagrammatically the difference between measured SCC in the sac technique and true SCC defined as the current required to reduce transepithelial PD to zero. The bladder itself is considered as three equivalent parallel paths, each comprised of a voltage source, E_{Na}, and a resistance, R_b. One path is close to the external current-passing electrode, one on the opposite side of the sac, and one in between. PD-monitoring electrodes are located in the middle path, so that apparent SCC, I, is the current required to reduce this PD to zero. The resistance of the external medium between the current-passing electrode and the bladder consists of r_1, measured between the electrode and the nearest portion of the tissue, and r_2, measured to the opposite portion. The middle path is halfway between. The "true" SCC is $3E_{Na}/R_b$. Thus, I overestimates SCC by $p - 1 + (p + 1)^{-1}$, where $p = r_2/2R_b$;

was investigated in four ways: (*a*) theoretically; (*b*) by moving the electrodes and observing the effects on SCC; (*c*) by observing the time course of the changes in SCC after changes in volume; and (*d*) by comparing SCC with net sodium flux before and after volume changes. The first two points are considered in Methods, and the last two in Results.

(*a*) Theoretical effects of volume changes on apparent *SCC*. To measure SCC ideally, the potential-monitoring electrodes should be an infinitesimally small distance from the two surfaces of the tissue, and the electrical field should be uniform over the surface of the tissue. In practice, this goal is unattainable and an error is present in any such device, although it may be negligibly small. The error is caused by an "IR drop" occasioned by the finite resistance, R, of the medium between the potential-measuring electrodes and the tissue. Thus, when the potential difference across the tissue is zero, there is still a potential difference between the electrodes given by the product of current, I, and R. If the geometry of the electrical field remains the same when the tissue is removed, this resistance is readily measured simply by passing current and measuring potential. The error can then be eliminated by balancing the potential difference not to zero, but to IR. I is then equal to the true SCC. However, if the geometry of the field is changed when the tissue is removed, no such correction can be made. In chamber experiments it is usually assumed that the geometry of the field is unaltered by removing the bladder. Strictly speaking, this would be the case only if the current-passing electrodes were planes parallel to and having the same area as the tissue. In practice, they can be so considered, provided that the distance between the electrodes and the tissue is large compared with the largest dimension of the tissue surface. The IR drop is then proportional to the distance between the potential-measuring electrodes and the tissue. If the current-passing electrodes are close to the tissue, the resisance, R_c, of the pathways which pass close to the center of the chamber is significantly less than the resistance, R_p, of pathways near the periphery. Thus, different IR drops are observed depending upon whether the potential-measuring electrodes are near the center or near the periphery. Furthermore, this variation is exaggerated in the process of measurement, when the tissue is removed, because the ratio R_c/R_p is lower than the ratio $(R_c + R_b)/(R_p + R_b)$, where R_b is the resistance of the tissue.

In the sac method used here, a single external electrode is used. Consequently the resistance, R_c, of the current pathway to the portion of the sac closest to the electrodes is lower than the resistance, R_p, of the pathway to the opposite surface of the sac. The difference, $R_p - R_c$, is the resistance of the serosal path between these points. This resistance, designated r_2, was measured with a bladder sac in place by observing the resistance of a pair of silver–silver chloride electrodes placed very close together and then placed on opposite sides of a bladder sac mounted in Ringer's solution. The difference in resistance was 12 ohms and was little affected by changing the volume of the sac. The resistance of the bladder sacs themselves averaged about 250 ohms and was volume dependent (see Results). The internal potential-measuring electrode, at the top of the sac, is midway between the current-passing electrode and the opposite side of the sac. An approximately equivalent circuit is shown in Fig. 1. The apparent SCC, I, is the current required to reduce the voltage, V, to zero. This can be readily calculated to be $I = [2 + p + 1/(p + 1)] \cdot E_{Na}/R_b$, where $p = r_2/2R_b$, whereas the true SCC should be $3E_{Na}/R_b$. Thus, I overestimates

SCC by $p-1+(p+1)^{-1}$. The overestimate increases when the bladder is stretched because R_b falls and p increases. For example, using $r_s = 10\ \Omega$ and $R_b = 250\ \Omega$ at 10 ml sac volume, the overestimate should increase from 0.4% to 1.1% on increasing the bladder sac volume from 10 to 30 ml, because R_b falls from 250 Ω to about 150 Ω (see Results).

(*b*) *Effects of moving the electrodes.* The open-circuit potential of the bladder was unaffected by moving either serosal or mucosal electrodes, as would be expected, but the apparent SCC was affected by certain maneuvers. Moving the two external electrodes about in the serosal bath had little or no effect until they came within 2 cm or less of one another. Apparent SCC then fell as they were brought closer together. In practice, the potential-measuring electrode was kept close to or touching the serosal surface, and the current-passing electrode was kept several cm distant. Moving the mucosal potential-measuring electrode up and down in the cannula had no effect on SCC, but when it was pushed down into the sac, SCC fell. It was therefore kept within the cannula. The position of the mucosal current-passing electrode was the only problem. The apparent SCC increased as this electrode was moved down from the geometric center of the approximately spherical sac toward the bottom, and increased as it was raised above this point toward the cannula. The change in apparent SCC per centimeter moved was greater when sac volume was small than when it was large. In a 30 ml sac, for example, the apparent SCC changed 4% on raising or lowering the electrode 1 cm, but in a 10 ml sac it changed 15%. For a uniform electrical field within the sac, the electrode should be at its geometric center—a point which obviously changes when sac volume is varied. In practice, this electrode was not moved unless the volume change was large (fourfold or more); under these circumstances, it was moved to the center of the sac.

Sodium flux measurements

Sodium fluxes were measured with ^{22}Na, ^{24}Na, or both. ^{24}Na was counted at an energy range of 3–4 Mev, and ^{22}Na 2 wk later at 1.2–1.4 Mev. Appropriate corrections were made for background and physical decay.

Because of the relatively large volume of bathing solutions, serosal/mucosal or mucosal/serosal isotope concentration ratios were very small (less than 0.02) and sample sizes were relatively large (0.2–1.0 ml). The quantity of sodium transported at each sampling time was calculated from the isotope concentrations, taking into account the quantity of isotope and volume removed with each sample. Three to six such values at 10-min intervals were obtained during each experimental period. Sodium flux was calculated from these values and the times by least square regression.

Wet weights and dry weights of the bladders after blotting were obtained at the end of each experiment.

Surface area, A, of the bladder was approximated from the volume of contained fluid, V, by $A = 3(3/4\pi)^{-1/3}V^{2/3}$ as would be the case for a perfect sphere. The error introduced by assuming spherical geometry is small compared with the error attributable to neglecting microscopic and submicroscopic irregularities of the mucosal surface. In chamber experiments, the same error is present and, in addition, gross foldings attributable to nonuniform wall tension are also neglected. Surface area as calculated here may thus be taken as equal to or slightly smaller than the area estimated in chamber experiments, for identical tissues; both estimates are some function of the true surface area. Little is known of this function, but it can safely be assumed to be monotonic.

Theoretically, the electrical conductance of an elastic spherical sac filled with and surrounded by highly conductive fluid increases with its volume. If the material of which the sac is composed is homogeneous, of unit density and volume V_b, the relationship between conductance, R^{-1}, and the volume of fluid in the sac, V_w, can be derived as follows:

The resistance R of a solid conductor is proportional to its length, r, and inversely proportional to its area, A. Therefore,

$$R = K^{-1}\int_{r_1}^{r_2} \frac{1}{A}\, dr,$$

where r_1 and r_2 are the internal and external radii respectively of the sphere, and K is the specific conductance in $ohm^{-1}\ cm^{-1}$. For a sphere, $A = 4\pi r^2$.
Integrating,

$$R^{-1} = \sqrt{2}\pi K(r_1^{-1} - r_2^{-1})^{-1}.$$

But

$$V_b = \tfrac{4}{3}\pi(r_2^3 - r_1^3), \text{ and } V_w = \tfrac{4}{3}\pi r_1^3$$

Hence,

$$R^{-1} = \sqrt{2}\pi(\tfrac{3}{4}\pi)^{1/3}K[V_w^{-1/3} - (V_b + V_w)^{-1/3}]^{-1}$$

The relationship between conductance and sac volume given by this equation is an approximately linear function of the square of surface area, as shown in Fig. 2. It should be noted that this analysis assumes uniform thinning of the membrane, whereas unfolding or enlargement of intercellular channels is also plausible.

Conductance of the bladders could be calculated as SCC/PD (potential difference) because the current-voltage curve of the tissue was linear in this range (4).

RESULTS

The temporal course of the response of SCC to change in volume

A representative experiment is illustrated in Fig. 3. Volume was increased from 10 to 40 ml in a period of 1 min. SCC rose steeply at first and then more gradually, to reach a maximum 80% higher than the control SCC. It remained nearly constant until volume was reduced, when it fell, though not to the original value. The sustained character of these responses is illustrated in Fig. 4, which shows, on a longer time scale, one of many experiments in which two volume changes were made in a period of several hours. In some bladders, such as the one illustrated, a progressive, slow decline in SCC began after a few hours, on which volume-induced changes in SCC could be superimposed for several more hours. The time course of the response to volume change in the first few minutes is of special interest, as it may shed light on the mechanism of the effect. As Fig. 5 shows, two or threefold volume changes had no immediate effect on SCC in most instances. Larger volume changes produced artefactual changes, as discussed in Methods. The observation shown in Fig. 5 constitutes strong evidence that the SCC changes recorded in response to smaller

volume changes were not electrical artefacts, since any such phenomena should be recorded as quickly as the recorder can respond—a matter of less than 1 sec with the equipment used. The lag period before SCC began to respond was distinctly longer with volume increases, where it varied from ½ min to 5 min, than with volume decreases. In the latter, a slow decline in SCC usually began coincident with volume change, and about 1 min later, the rate of fall increased to a maximum.

The rate of change of volume altered the time course of the response, but not the final response achieved, except when volume was increased very rapidly (in less than ½ min). When this was done, SCC usually fell at first and then gradually rose to a value above the control level, but less high than with slower changes. Rapid volume decreases, however, produced the same final response as slower ones.

Figs. 6 and 7 summarize experiments in normal Ringer's solution with glucose in which two- to fourfold volume changes were produced over 1–3 min. Most of these were conducted with an open cannula and some with a plug (see Methods). Because the results were not significantly different in any of the time intervals shown, they have been combined.

As the figures show, the magnitude of the response varied considerably among experiments. Occasional bladders exhibited virtually no change in SCC after repeated volume changes. These experiments, though few in number, establish that the response is not an inevitable consequence of some feature of the experimental design or the apparatus employed.

The reduced SCC following volume decrease was almost always sustained, but the increment following a

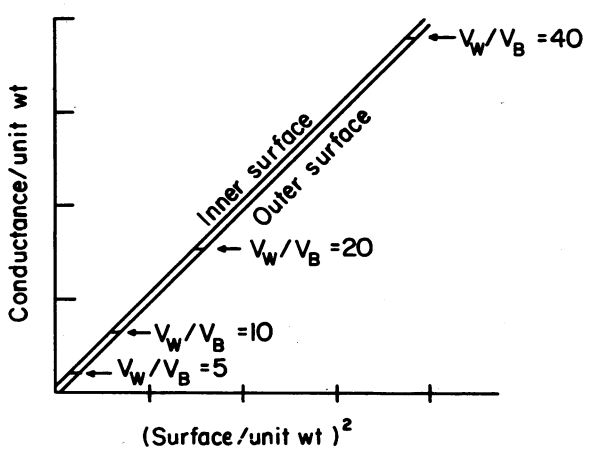

FIGURE 2 Theoretical relationship between conductance and the square of surface area of a hollow elastic sphere when its volume is varied. The function is approximately linear, in terms of inner or outer surface of the sac. V_W = volume of fluid within the sac; V_B = volume of the material of which the sac is composed.

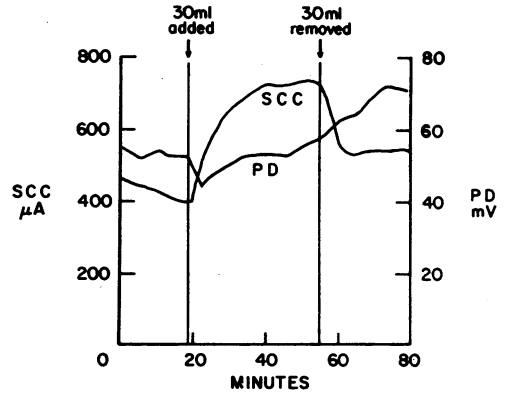

FIGURE 3 Effect of volume changes on SCC and PD in a representative experiment. PD was measured every 190 sec.

volume increase often reached a peak at about one-half hour, and then fell somewhat (Fig. 6). This may be a reflection of the fact that baseline SCC in this preparation often declines after the first few hours.

Graded character and reversibility of the response to stretch. In individual bladders, the magnitude of the change in SCC was dependent upon the magnitude of the volume change. Successive increases in SCC could be produced by successive additions of fluid, and conversely. To compare the results from different experiments, surface area per milligram wet weight was estimated from sac volume, as discussed in Methods. This parameter, which is a measure of stretch, could be varied over a range of approximately 0.03–0.4 cm^2/mg. The lower limit was determined, not by the ability of the bladder to contract, as explained below, but by the apparatus used, because the reliability of SCC measurements diminished as the distance between current-passing and potential-measuring electrode decreased. The upper limit

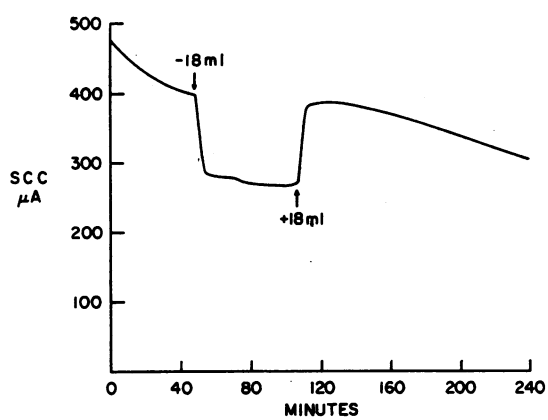

FIGURE 4 Responses to removal and addition of 18 ml of fluid (in less than 1 min) to a bladder sac initially containing 4 ml. The baseline SCC is declining throughout the course of the experiment.

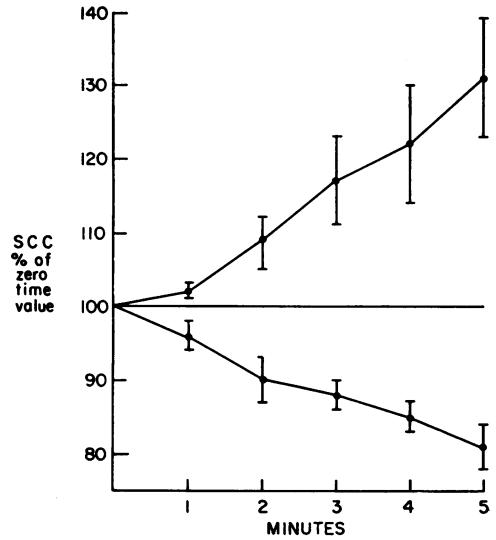

FIGURE 5 Early course of the response of SCC to volume changes. At time zero, a two- to fourfold increase or decrease in volume was made during approximately 1 min. The vertical bars represent 2 × SEM.

in open cannula experiments was determined by the amount of fluid the bladder would accept at the pressure dictated by the cannula height (4 cm), and in closed cannula experiments by the maximum amount of fluid the sac could hold without bursting. This latter limit was measured in several bladders, and averaged 0.35 cm^2/mg. In most experiments the upper part of the range of stretch was not employed, so as to avoid rupture. If tissue density is taken as unity, the stretch range of 0.03–0.4 cm^2/mg corresponds to an epithelial thickness range of 25–333 μ.

SCC per milligram wet weight, at 30 min after a volume change, is dependent upon the degree of stretch. In many individual bladders the relationship between these two variables was linear, with a positive intercept on the SCC axis. However, a drifting baseline SCC often obscured the linearity in such a plot. Consequently, the mean slope between the SCC immediately before and 30 min after volume change was calculated for each experiment. Increases and decreases were considered separately. A weighted mean slope for all experiments was calculated, as well as a weighted standard error (Table I).[1] The response in 35 experiments averaged 10.6 ±1.3 $\mu a/cm^2$ for increases and 10.1 ±1.3 $\mu a/cm^2$ for decreases. The similarity of these values shows that the phenomenon is fully reversible. There was no correlation between the slope of SCC response and the magnitude of volume change ($r = -0.071$). This finding further supports the conclusion that the magnitude of the response, in microamperes per square centimeter, was determined by the magnitude of the change in surface area.

[1] $\bar{x} = \Sigma n_i x_i / \Sigma n_i$, where x_i is the mean slope for the i^{th} hemibladder and n_i is the number of volume changes. $\sigma_{\bar{x}}^2 = \Sigma n_i (x_i - \bar{x})^2 / \Sigma n_i (N-1)$, where N is the number of hemibladders (5).

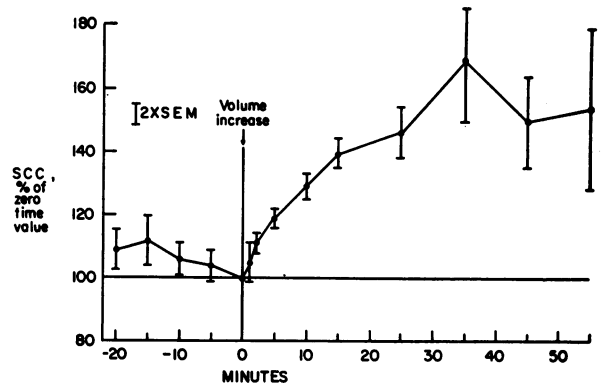

FIGURE 6 Time course of SCC response to volume increase in control experiments. Volume was increase two- to fourfold within 1 min at time zero.

The over-all mean slope, 10.4 ±0.9 $\mu a/cm^2$, indicates that an increase in surface area from 0.03 to 0.2 cm^2/mg would increase SCC by 1.8 μa/mg. When expressed per unit area however, SCC is regularly reduced by stretching because the extrapolated intercept value of SCC (at "zero stretch") is always positive.

Sodium flux measurements

The correspondence between net sodium transport and SCC was determined in 19 experiments. The former averaged 99 ±7% (SEM) of the latter. The results indicate that SCC is a measure of net sodium transport in this preparation, as in conventional chamber experiments, when using Ringer's solution. Passive backflux of sodium was relatively low, averaging 0.45 ± 0.006 μa/mg wet weight (SEM, $n = 42$). The ratio of "active" (mucosal to serosal) flux to backflux was high in comparison with values we have previously observed in chambers; the mean was 13.5 ($n = 19$) with a range of 2.2–86. Passive sodium backflux was correlated linearly with conductance (Fig. 8).

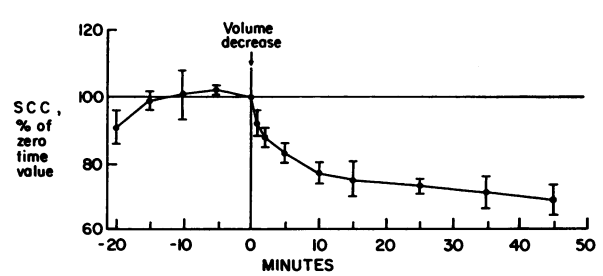

FIGURE 7 Time course of SCC response to volume decrease in control experiments. Volume was decreased two- to fourfold in about 1 min.

TABLE I

Stretch Responses of Toad Hemibladders, Measured 30 min after Volume Change

Group and No. of experiments	Description	Weighted mean change in SCC		
		Increases	Decreases	Combined
			$\mu A/cm^2$	
I (21)	Controls	9.6 ±1.5* (36)‡	9.6 ±1.4* (21)‡	9.6 ±1.1* (57)‡
II (14)	Minus glucose	12.7 ±2.1 (18)	10.3 ±3.1 (9)	12.1 ±1.7 (27)
III (35)	I plus II	10.6 ±1.3 (54)	10.1 ±1.3 (30)	10.4 ±0.9 (84)
IV (5)	Sulfate Ringer's	8.8 ±2.5 (7)	10.0 ±4.8 (6)	9.1 ±2.4 (13)
V (5)	Everted	5.7§ ±1.4 (9)	6.8 ±1.3 (8)	5.8‖ ±0.9 (17)

* Weighted standard error.

‡ Number of volume changes recorded.

§ Significantly different from Group III ($P < 0.01$).

‖ Significantly different from Group III ($P < 0.001$).

The effect of stretch on sodium flux from mucosa to serosa was evaluated in 14 experiments. In every case, sodium efflux changed in parallel with SCC. Net sodium flux change and SCC change were compared in seven experiments. The correspondence is illustrated in Table II.

Fig. 8 also shows that sodium backflux increased only irregularly in response to stretch. As indicated in the next section, conductance always varied with stretch. In one bladder, a large increase in conductance and sodium backflux occurred, in the absence of a leak. In the other experiments, the effects of volume change on sodium backflux were variable, though usually in the same direction as the change in volume and in conductance.

Effect of stretch on transepithelial potential difference (PD) and conductance. The range of PD's observed in those experiments was 25–140 mv. Most values were between 40 and 110 mv. As illustrated in Fig. 4, a change in PD coincident with volume change but opposite in direction often occurred. The magnitude of this change varied with the magnitude of change in volume. After volume change, PD tended to return towards (but not to) its value before volume change, particularly when SCC responded vigorously. The final value at which conductance (calculated as SCC/PD) stabilized was always higher for volume increases, and lower for volume decreases, and the change in conductance was almost always greater than the change in SCC. In other words, the PD rarely returned to its control value.

To express these results quantitatively, we have related the conductance per milligram wet weight, determined 30 min after volume change, to the degree of

TABLE II

Comparison of Stretch-Induced Changes in SCC with Simultaneous Changes in the Net Transepithelial Flux of Sodium

Experiment No.	Change in stretch	Change in SCC, a	Change in net J_{Na}, b	Difference |a| − |b|
	cm^2/mg	$\mu A/mg$	$\mu A/mg$	
290	+0.058	+0.43	+0.33	+0.10
298	+0.091	+1.40	+1.76	−0.36
321	−0.082	−0.84	−0.83	+0.01
338	+0.072	+0.35	+0.37	−0.02
352	+0.079	+0.78	+0.74	+0.04
379	+0.064	+0.04	+0.31	−0.17
393	−0.148	−1.83	−1.53	+0.30
				−0.01 ±0.08 (SEM)

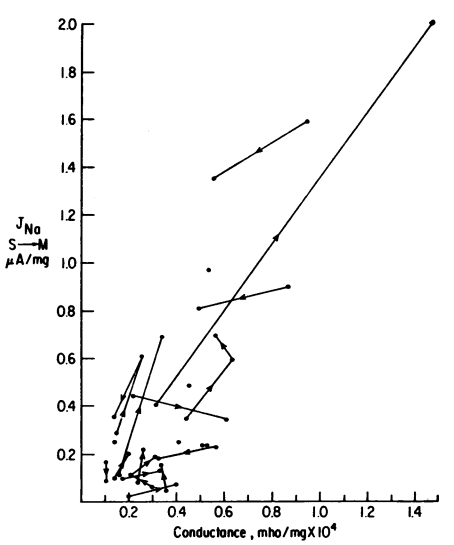

FIGURE 8 Serosal-to-mucosal sodium flux in short-circuited hemibladders as a function of conductance, calculated as SCC/PD. Observations in single experiments in which volume was changed are connected by lines. Conductance change always reflects change in stretch but sodium flux change does not do so consistently.

stretch, expressed as surface area per milligram wet weight. Since the conductance of a hollow sphere composed of homogeneous elastic material increases approximately as the square of its surface area (see Methods), bladder conductance has been related to the square of stretch. Fig. 9 shows the results in several representative experiments. In each case, a linear relationship is seen, but a positive intercept is present varying from 0.0001 to 0.0003 mho/mg wet weight.

To determine whether this relationship depends upon the stretch response, SCC and PD were reduced to zero by the addition of 10^{-4} M dinitrophenol and 10^{-3} M ouabain to the serosal bath. Conductance was then determined from the current required to produce a PD of 50mv (serosa positive). The relationship between conductance and $(\text{stretch})^2$ was similar to that seen in Fig. 9 and thus this phenomenon is not dependent upon the presence of active sodium transport.

Effects of pressure on SCC. Nutbourne (6) has recently reported an effect of small changes in hydrostatic pressure on SCC in the frog skin and has shown that stretching the tissue mechanically, without pressure change, fails to elicit this response. This seemed an unlikely explanation of the phenomenon under study here because of the peculiar characteristics of the pressure-volume relationship in this organ.The bladder exhibited spontaneous contractions which varied pressure from 1 to 4 cm H_2O without having any effects on SCC. These contractions were most evident in sacs containing smaller amounts of fluid. They occurred more or less regularly at intervals of about 1 min. In the open-circuit state, they were accompanied by simultaneous changes in PD, opposite in direction. In the closed circuit state, however, SCC did not change. Furthermore, there is a

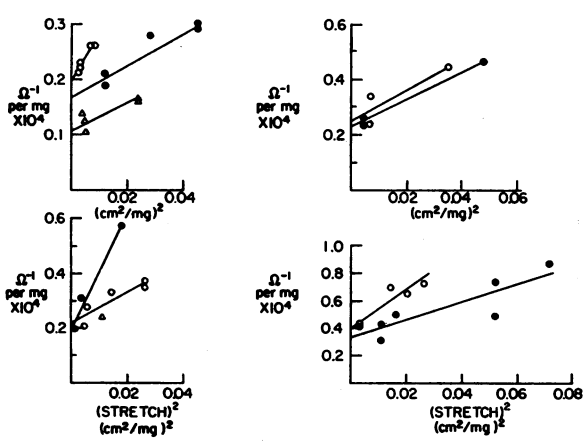

FIGURE 9 Bladder conductance as a function of the square of stretch in representative experiments. Results in nine hemibladders are shown on four graphs for clarity. The relationship is linear in all, but the slope and intercept vary considerably. All SCC's measured 30 min after volume change.

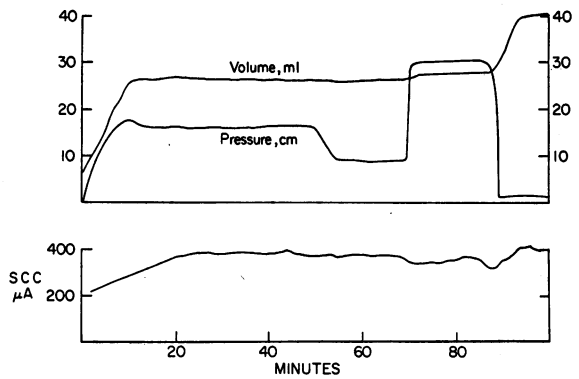

FIGURE 10 SCC response to pressure. The hemibladder was enclosed in a nylon net. Filling the sac increased volume, pressure, and SCC. Subsequent variations in pressure did not affect volume or SCC. When the net was removed, pressure fell to its normal value, volume rose, and SCC rose slightly.

surprising degree of independence of pressure and volume in these sacs, provided that volume is relatively small. Volume can be changed only by altering pressure as fluid is added or removed, but within a few minutes the mean pressure is back to almost the same value. However, at higher degrees of stretch, pressure rises appreciably. Rupture occurs at about 25 cm H_2O.

To investigate the role of pressure further, we enclosed bladders in nylon nets. The cannula was fitted with a plug (see Methods) with a single inflow tube. By varying the height of the fluid-filled reservoir, pressure could be varied at will. The results of one such experiment are shown in Fig. 10. Raising the reservoir at first led to an increase in volume as well as SCC, but subsequent increments in pressure had no effect on either. When the nylon net was removed, pressure fell but volume and then SCC rose. The results in four other experiments were essentially the same. Furthermore, as shown below, everted bladders, in which the pressure gradient is reversed, nevertheless respond to stretch. Thus, pressure is not responsible for the SCC changes under study here.

Effect of the absence of glucose. In 14 experiments, glucose was omitted from the medium to determine whether the response is dependent upon exogenous substrate. These experiments were run concurrently with experiments in the presence of glucose on toads from the same batches. The results are shown in Figs. 11 and 12. The response to volume increases is significantly greater (by Student's t test) at 5, 10, 15, and 25 min, but not at later times. At every time interval tested, the fall in SCC in response to volume decreases is greater in the absence of glucose than in its presence. However, none of these differences is individually significant. The difference diminishes with time and is only 1% at 35

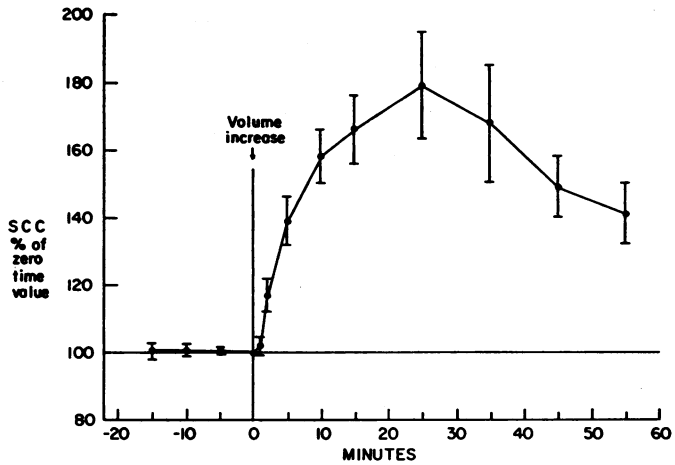

FIGURE 11 Time course of SCC response to volume increase in glucose-free medium. The response is faster than in the control experiments (Fig. 6) and reaches a higher peak (at 25 min), but 35-min values are the same.

min. The weighted average slope of SCC change in the absence of glucose, measured at 30 min, is not significantly different from that of control with glucose (Table I). Thus, the absence of glucose does not alter the magnitude of the final effect but increases the speed of response.

Effect of the absence of chloride. The possible role of alterations in chloride permeability was examined by conducting experiments in Ringer's solution in which all of the chloride was replaced by sulfate.[2] The results were unaltered both in terms of the time course of the response and the 30 min value (Table I). Thus, the presence of chloride is not a necessary condition for the response.

Responses in everted bladders. When the bladders were everted before mounting, the responses to volume change were distinctly diminished (Table I), both to increases and decreases. The combined responses are significantly diminished at the $P < 0.001$ level. Nevertheless, the pattern of response was qualitatively the same.

Effect of 100% oxygen. One possible explanation of the stretch response might be that access of oxygen to the mucosal epithelium is diffusion-limited, and hence might vary with the extent to which the tissue is stretched. If oxygen access were diffusion-limited and were a determinant of SCC, an increase in oxygen concentration of the medium, in the absence of stretching, should increase SCC.

The effect of changing from air to 100% oxygen in the bubbling tubes in both serosal and mucosal media is illustrated in Fig. 13. No stimulation of transport occurred; in fact, there was a significant decline in SCC. Conceivably, the decline may have been unrelated to oxygen administration. Because the purpose of these experiments was merely to establish that oxygen was not stimulatory, this phenomenon was not investigated further.

DISCUSSION

These experiments demonstrate that the toad bladder possesses an intrinsic mechanism for altering sodium transport in response to stretch. Several considerations militate against the possibility that these responses are artefactual: (*a*) the artefactual change in SCC predicted on theoretical grounds is small in comparison with the effects observed; (*b*) the response occurs subsequent to the change in volume; (*c*) in occasional bladders, no response occurs; and (*d*) the changes in net sodium flux are comparable.

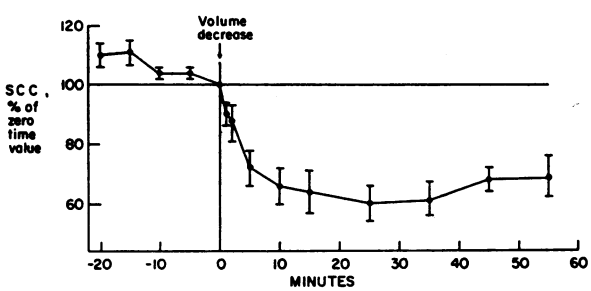

FIGURE 12 Time course of SCC response to volume decrease in glucose-free medium. As in Figure 11, the response appears to be faster than in the control experiments (Fig. 7), but reaches the same final values.

[2] 55 mM Na_2SO_4, 3 mM $NaHCO_3$, 3 mM K_2SO_4, 1 mM Ca acetate, 1 mM $MgSO_4$, and 5.5 mM glucose.

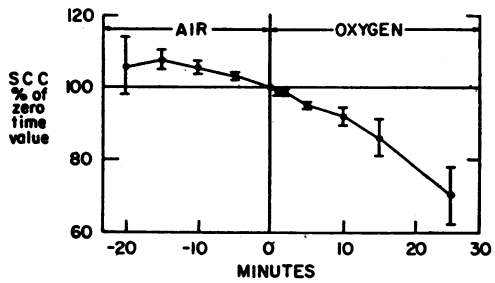

FIGURE 13 The effect on SCC of gassing the medium with oxygen (100%) instead of air. A fall in the SCC ensues. Volume was kept constant.

The sac technique imposes some special problems; the most important are those of electrode placement with its associated artefacts, and the large bath volumes required with associated difficulties of flux measurements. On the other hand, stretch can be controlled, larger amounts of tissue can be examined, and the epithelium exhibits a considerably lower passive ionic permeability. This is borne out by relatively high PD, low conductance, and low sodium backflux seen in these experiments in comparison with previous observations using conventional chambers, by us or by others (2, 3). Sacs are widely used for the measurement of water permeability (3, 7), but have only occasionally been employed for electrical measurements on this tissue (8). Turtle bladders mounted as sacs exhibit high PD's and transport sodium at a high rate relative to their weight (9).

The higher ionic conductance in chamber experiments may be attributable to the "edge damage effect" recently described in frog skin (10). The PD of this tissue was shown to increase as the edge-to-surface ratio of the chamber decreased. The lowest ratio attainable in conventional chambers is obtained with the largest chamber, conceivably a circle of 5 cm radius for the toad bladder. Such a chamber would have an edge-to-surface ratio of 0.4. But to mount a bladder in such a chamber it would certainly be necessary to stretch it, which in itself would increase conductance. An unstretched bladder could not readily be mounted in a chamber with a ratio lower than 2. In the sac technique used here the edge is constant at 3 cm and the surface can be varied from 10 to 100 cm^2, with corresponding ratios of 0.3 to 0.03. It is conceivable that much of the ionic conductance in chamber-mounted bladders is at the damaged edge.

The effect of stretch on conductance (Figs. 9 and 10) may also bear some relation to edge damage effects. The positive intercepts suggest that some portion of the tissue is not increasing its conductance in response to stretch, i.e., is not stretching. Whether this represents damaged edge, intercellular spaces, cell membranes, or other components remains to be determined.

The mechanism of the stretch response is not shown by these experiments, but evidence bearing on several possibilities has been presented. The most obvious mechanism is the conductance change itself. This effect of stretch never fails to occur; it precedes and is greater in magnitude than the change in sodium transport. It is not obvious, however, that an increase in sodium transport can or should result. If access of sodium to the transport mechanism is rate-limiting (3), increased ionic conductance might mean a greater rate of entry of sodium into the transport pool. The rate of turnover of the active transport pool of sodium, based upon our observed values of SCC and reported estimates of the pool size (3) should be the right order of magnitude to produce the speed of response which was observed. However, there is conflicting evidence on this basic premise (11). Furthermore, serosal-to-mucosal sodium flux, which might provide a measure of the access of sodium to the pump (provided it reflects accurately the active transport path), did not consistently vary with stretch. Nevertheless, this postulated mechanism remains tenable until further evidence is obtained.

Increased access of substrate or of oxygen due to thinning of the epithelium has been excluded as an explanation of the results as has increased permeability to chloride.

Some of the more attractive possibilites which remain to be examined are (*a*) altered geometry of intercellular spaces, (*b*) increased rate of synthesis of adenosine triphosphate (ATP), and (*c*) increased activity of sodium-potassium–activated ATPase. The latter two effects could result from conformational changes in enzyme proteins which are related to alterations in membrane tension or from altered rates of enzyme synthesis.

It also remains to be determined whether this mechanism, intrinsic to the toad bladder, is peculiar to this organ and species. The only relevant experiments appear to be those of Nutbourne (6), who found a pressure response in frog skin, but no effect of stretch. The validity of her stretch experiments may be questioned, however, since the mechanical device used to stretch the skin could have injured it and obscured the response. The relationship of the phenomenon observed here to the renal tubule is uncertain. In some studies (12, 13), a relationship between tubular reabsorption of sodium and tubular diameter has been found, but in other reports (14, 15) no such relationship has been observed. Conceivably, the renal tubular epithelium possesses this mechanism, but the response is modified by constituents of the plasma or urine. The present technique offers a model system to test for such constituents, but experiments on intact nephrons will be necessary to determine their relevance.

In the toad, a role of this mechanism in salt balance is conjectural. However, the bladder does reabsorb a significant proportion of the salt excreted by the kidneys during salt restriction (16). More important, the similarity of the toad bladder epithelium to that of the distal nephron suggests that the same mechanism may exist in the toad's kidney, where it might play a significant role in glomerulotubular balance.

ACKNOWLEDGMENTS

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