J. Membrane Biol. 28, 295–307 (1976)
© by Springer-Verlag New York Inc. 1976

Sodium Transport by the Colon of *Bufo Marinus:* Na Uptake Across the Mucosal Border

David C. Dawson* and Peter F. Curran**

Department of Physiology, Yale University, School of Medicine,
New Haven, Connecticut 06510

Received 21 January 1976; revised 5 April 1976

Summary. Na transport by the isolated toad colon has been studied by measuring transmural Na fluxes and by direct measurement of the Na influx across the mucosal border. Net Na transport accounts for 88% of the short circuit current in the presence and in the absence of exogenous aldosterone. Na influx across the mucosal border appears to consist of two components. One component is highly correlated with short circuit current, is a saturable function of mucosal Na concentration, and is inhibited by lithium ions in the mucosal medium. The second component is a linear function of mucosal Na concentration, is unaffected by lithium, and is apparently not related to net Na transport by the tissue.

The isolated colon of *Bufo marinus* can transport Na actively [5], but little is known about the details of ion movement across this tissue. A simple, three-compartment model for transepithelial ion transport suggests that active Na transport represents the movement of Na across at least two barriers in series, the membranes at the two sides of the epithelial cell layer. Direct measurement of Na entry into epithelial cells of rabbit ileum [13] and frog skin [1] has indicated that Na may enter these cells by interacting with some component of the cell membrane. The present experiments were undertaken in order to obtain additional information as to the nature of Na transport by the toad colon[1]. Trans-epithelial, unidirectional Na fluxes were measured in the presence and absence of exogenous aldosterone and the technique of Biber and Curran

* *Present address*: Department of Physiology and Biophysics, University of Iowa, College of Medicine, Iowa City, Iowa 52242.
** The original draft of this manuscript was reviewed by Dr. Curran before his untimely death on October 16, 1974. It is hoped that the final version fairly reflects his views, as well as those of the first author.
1 A preliminary report of these experiments was presented at the 1974 meeting of the Biophysical Society (*Fed. Proc.* **33**:1514, 1974).

[1] was employed to measure directly the unidirectional influx of Na from the mucosal medium into the cellular compartment of the colon. The results suggest that Na entry into the cells may proceed, at least in part, by a saturable, Li inhibitable, influx process.

Materials and Methods

Preparation of the Colonic Mucosa

All studies were performed on colons obtained from female *B. marinus* of Colombian origin (Tarpon Zoo, Tarpon Springs, Florida) which were housed on moist wood chips for one to ten days prior to an experiment. For all experiments, the colon was first stripped of its longitudinal and circular muscle layers according to the technique of Parsons and Patterson [11] as described by Lew [9]. Animals were pithed and the colon was quickly removed through a ventral incision. The proximal end of the colon was tied on to a length of polyethylene tubing connected to a syringe, and ice cold Ringer's solution was flushed through the colon to remove fecal matter. The distal end was tied off and the resulting sac inflated with Ringer's and maintained under moderate tension during the dissection. A longitudinal incision was made along the length of the distended colon with a razor blade, and the muscle layers were peeled off. Histological examination of tissues prepared in this manner showed a single layer of epithelial cells on the mucosal side with a thin *muscularis mucosae* and a loose layer of connective tissue remaining on the serosal side.

Transepithelial Unidirectional Na Fluxes

The mucosa, stripped of its muscular layers, was laid out as a flat sheet and divided lengthwise so that paired sheets could be employed for flux measurements. Portions of the mucosa were mounted in chambers similar to those described by Schultz and Zalusky [14] which were equipped with 4 Ringer-agar bridges, two connected to calomel half-cells for the measurement of the transepithelial potential difference (*PD*) and two connected to Ag–AgCl electrodes for passing current across the tissue. The area of exposed tissue was 1.13 cm^2. The *PD* was maintained at 0 mV by an automatic voltage clamp device which compensates for the solution resistance between the *PD* electrodes and the tissue surface. Both sides of the mucosa were bathed by identical solutions containing 112 mM NaCl, 2.5 mM $KHCO_3$, 1.0 mM $CaCl_2$, 5.0 mM D-glucose and 2.5 mM Na pyruvate. Each solution was stirred and oxygenated by a stream of air bubbles, yielding a pH of 8.1. The temperature of the solutions was regulated at 25 °C.

Mucosa to serosa and serosa to mucosa unidirectional Na fluxes were measured simultaneously under short circuit conditions using ^{22}Na and ^{24}Na as tracers. 5.0 μC of ^{22}Na was added to the mucosal solution (15 ml) and 30 to 50 μC of ^{24}Na to the serosal solution (15 ml) and one hour was allowed to establish steady-state tracer fluxes. 1.0 ml samples were taken from both sides at 1 hr intervals thereafter for 8 hours. Samples from the mucosal side were added to 15 ml of water and counted immediately for ^{24}Na and ^{22}Na (Cherenkoff radiation) in a liquid scintillation spectrometer (Nuclear Chicago) and then counted again at least 14 days later for ^{22}Na in order to obtain the net activity of ^{24}Na in each sample. Samples from the serosal side were added to 10 ml of Bray's solution [3] and counted at least 14 days later for ^{22}Na. The short circuit current (I_{sc}) and open circuit *PD*

were recorded at the time of each sample. D-aldosterone (Sigma) was added to the serosal side of one of a pair of tissues at the time of the addition of isotope in 10 μl of methanol to achieve a final concentration of 10^{-6} M.

Unidirectional Influx of Na

The unidirectional influx of Na from the mucosal solution into the mucosal cell layer was estimated by measuring the uptake of ^{24}Na according to the technique of Biber and Curran [1]. For these studies individual discs of stripped mucosa of about 1 cm diameter were punched out. A disc of tissue was mounted in an influx chamber, similar to that described by Biber and Curran [1], and both sides of the tissue were bathed by identical solutions. The chamber was equipped with appropriate electrodes and connected to a voltage clamp so that the tissue could be maintained in the short-circuited state. After a stable I_{sc} was obtained, approximately 3.5 ml of a test solution containing Ringer's solution and 10 μC ^{24}Na and 50 μC ^{3}H-mannitol was injected into the chamber on the mucosal side of the tissue. After approximately 30 sec, the tissue was rapidly blotted and punched out of the chamber. The time of exposure was defined as the time from injection of the test solution to blotting of the tissue. The tissue was then extracted in 0.1 N HNO_3 and aliquots of the extract were assayed for ^{24}Na and ^{3}H. ^{3}H-mannitol served to estimate the amount of the test solution adhering to the mucosal after blotting. Recent studies on frog skin [2] have shown that mannitol equilibrates much more rapidly with this "extracellular space" than does inulin. The amount of ^{24}Na in excess of that in the "mannitol space" was used to calculate the unidirectional influx of Na into the tissue. Na concentrations in the test solutions were measured by flame photometry.

Results

Transmural Na Fluxes

The results of double-label Na flux experiments are summarized in Figs. 1 and 2 and Table 1. Fig. 1 shows the time course of the tissue response to aldosterone as indicated by a plot of the average I_{sc} normalized to the value at one hour. Both control and treated tissues exhibit a steady decline from an initially high value, the rate of decline being less in the treated tissues. Table 1 shows the average values of the fluxes and electrical parameters for both groups. In Fig. 2 the net Na fluxes for treated and untreated tissues during individual flux periods are plotted versus the corresponding I_{sc}. The solid line is the least squares regression line for these points. It has an intercept not significantly different from zero and a slope of 0.88 ± 0.03, a value significantly different from 1.0 ($p < 0.001$). If the data from treated and untreated tissues are plotted separately each plot exhibits a zero intercept and a slope of 0.88. This suggests that the relationship between the net Na flux and I_{sc} is similar for treated and

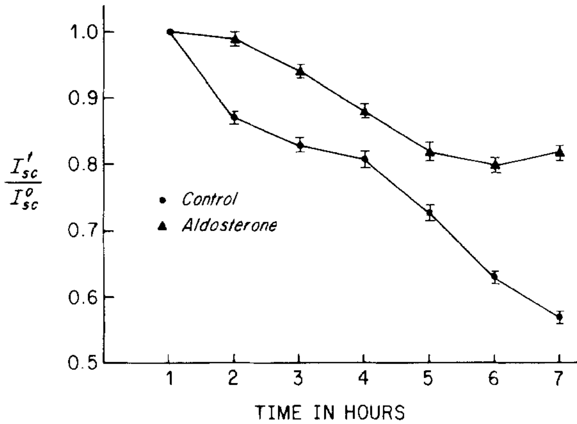

Fig. 1. The average I_{sc} (±SEM) for control and aldosterone-treated tissues normalized to the initial value for each group and plotted *vs.* time. The error bars are two standard errors of the mean (SEM)

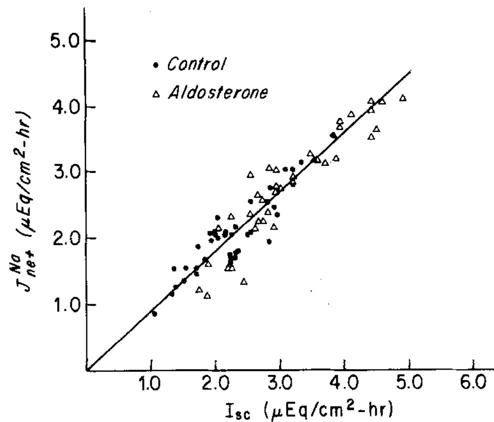

Fig. 2. Values of J^{Na}_{net} for individual 1-hr flux periods plotted as a function of I_{sc}

Table 1. Transmural Na fluxes

	J_{ms}	J_{sm}	J_{net}	I_{sc}	PD	G_m	n
		μEquiv/cm²-hr			mV	mmho/cm²	
Control	2.6±0.1	0.5±0.1	2.1±0.2	2.3±0.2	38.6±5.5	1.9±0.2	6
Aldo	3.5±0.3	0.6±0.1	2.8±0.3	3.2±0.4	47.6±7.6	2.2±0.3	6

untreated tissues, and that, on the average, about 88% of I_{sc} is composed of a net flux of Na from mucosal to serosa. This value is comparable to the 93% reported by Cofré and Crabbé [5].

Unidirectional Influx of Na

Measurements were made of Na uptake as a function of time of exposure to the test solution to determine the time during which Na uptake represents the unidirectional influx of Na into the tissue. In these experiments, the serosal solution was also sampled at the end of the influx period. Assay of these samples indicated that at times up to 60 sec there was virtually no transepithelial movement of tracer. A plot of Na uptake at 15, 30, and 60 sec is shown in Fig. 3. The points can be described by a straight line which extrapolates to the origin. This suggests that over a 60 sec period Na uptake is essentially unidirectional and that this method provides a reliable estimate of the unidirectional influx of Na from the mucosal medium into the tissue.

Measurements of Na influx were made at Na concentrations of 11.5, 25.8, 50.2, 81.5, and 109.8 mM in experiments utilizing 15 toads. In any individual experiment, Na influxes were measured at three Na concentrations, one of which was usually 109.8 mM as a control for animal to animal variation. In such experiments both sides of the tissue were bathed by identical Ringer's solution (*see Materials and Methods*) in which variable amounts of NaCl were replaced isosmotically by choline chloride. Fig. 4 shows the average Na influx, J_i^{Na}, and the average I_{sc} plotted as a function of Na concentration. The relation between J_i^{Na} and Na concentration appears to be nonlinear and the points can be described by a curve which

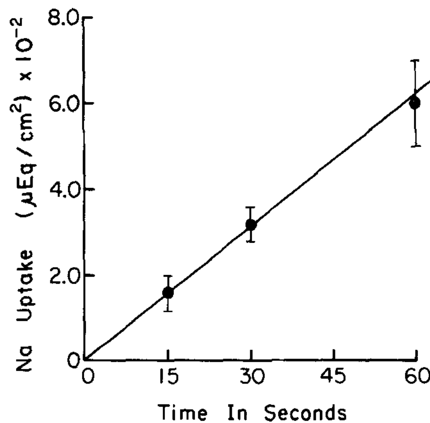

Fig. 3. Plot of Na uptake (±SEM) across the mucosal border of the colon as a function of time of exposure to the test solution. Each point is the mean of from 4 to 6 determinations on tissues from two toads

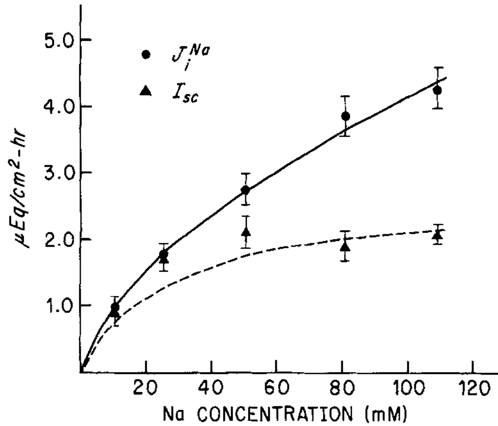

Fig. 4. Plot of J_i^{Na} (±SEM) and I_{sc} (±SEM) as a function of Na concentration. *See text* for details

consists of the sum of a saturating and linear component, i.e.,

$$J_i^{Na} = \frac{J^m [Na]_m}{K_{Na} + [Na]_m} + \alpha [Na]_m. \quad (1)$$

Values for J^m, K_{Na} and α were obtained by varying these parameters to obtain a minimum sum of squared deviations of the calculated J_i^{Na} from that actually observed. Error estimates for the parameters were obtained by first lumping all of the error into the saturating component, subtracting the linear component and calculating a least squares fit for the saturating component to a Scatchard plot [12]. Similarly, by lumping all of the error into the linear component, an error estimate for α was derived. The values thus obtained were $J^m = 2.8 \pm 0.2$ μEquiv/cm²-hr, $K_{Na} = 31.0 \pm 5.3$ mM, and $\alpha = 0.020 \pm 0.001$ cm/hr. The form of the saturating component of Na influx is indicated by the dashed line in Fig. 4. The fit to the data obtained by assuming that the curve consisted solely of either a simple linear or single saturating component was much less satisfactory. The apparent "linear component" can, of course, not be distinguished from a saturating component with a very high apparent Michaelis constant.

In the absence of additional information, the significance of the two apparent components of Na influx as defined by such a curve fitting procedure is not clear. It is possible, however, to obtain further insight into this problem by examining the relationship between Na influx and short circuit current at different Na concentrations. Fig. 5 is a plot of Na influx versus I_{sc} at an Na concentration of 25 mM. The line is the least squares fit to the points. J_i^{Na} exhibits a high correlation with I_{sc}, indicating

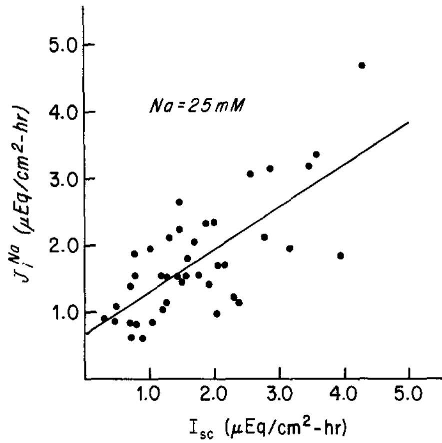

Fig. 5. Plot of J_i^{Na} *vs.* I_{sc} at 25 mM Na

Table 2. Least squares fit for J_i^{Na} *vs.* I_{sc} at different Na concentrations

[Na] (mM)	Slope	Intercept	r
109.8	1.14	1.90	0.41
81.5	0.77	2.44	0.37
50.2	0.74	1.15	0.76
25.8	0.63	0.70	0.73
11.5	0.85	0.22	0.89

that a portion of Na influx is related to active Na transport across the tissue. Table 2 lists the slopes, intercepts, and correlation coefficients for similar plots at each Na concentration. The lines have similar positive slopes, and positive intercepts which clearly tend to increase with increasing Na concentration. Note that the correlation coefficients for 109.8 and 81.5 mm Na are relatively low, indicating the large amount of scatter in these plots at high Na concentrations.

The relationship between J_i^{Na} and I_{sc} also suggests that the total influx of Na across the mucosal border of the colon consists of two components, one correlated with I_{sc} and one independent of I_{sc} but dependent on Na concentration, i.e., these relations are all of the form:

$$J_i^{Na} = m I_{sc} + \alpha [\text{Na}]. \quad (2)$$

In Fig. 6 the intercepts of the regression lines of Table 2 are plotted as a function of Na concentration. The least squares line through these points has an intercept not greatly different from zero and a slope of

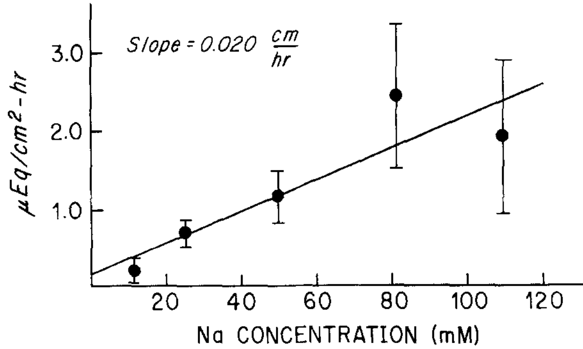

Fig. 6. Plot of the intercepts listed in Table 2, (±SEM) as a function of Na concentration

0.020±0.01. This suggests that the portion of the Na influx which is independent of I_{sc} can be adequately described as a linear uptake component with a permeability coefficient of 0.020. This value is identical to that obtained for the parameter α by curve fitting to the plot of J_i^{Na} versus Na concentration (Fig. 3) and suggests that α represents the permeability of a linear uptake component which is not correlated with net Na transport.

The average slope of the plots of J_i^{Na} versus I_{sc} is 0.86, a value close to the slope of 0.88 obtained from plots of the net Na flux versus I_{sc} for transmural experiments. This suggests that a plot of J_i^{Na} versus the net Na flux would have a slope close to 1.0. Since one attractive possibility is that the saturating component of Na influx is involved in the process of net Na transport by the tissue, it is of interest to compare the saturating component of Na influx with the I_{sc} measured in these experiments. Fig. 4 indicates that the saturating component of Na influx and the I_{sc} exhibit a similar dependence on Na concentration.

The Effect of Li on Na Influx

Biber and Curran [1] have shown that Na influx into frog skin is competitively inhibited by Li. Since the saturable form of one component of Na influx into the toad colon suggests the possibility of mediated entry process, some experiments were undertaken to test the effect of Li ions on Na influx. For these experiments an Na concentration of 25 mM was chosen to reduce the linear component of influx, and influx measurements were conducted exactly as those previously described, except that during the 30 sec exposure period the test solution contained 25 mM Na and 0,

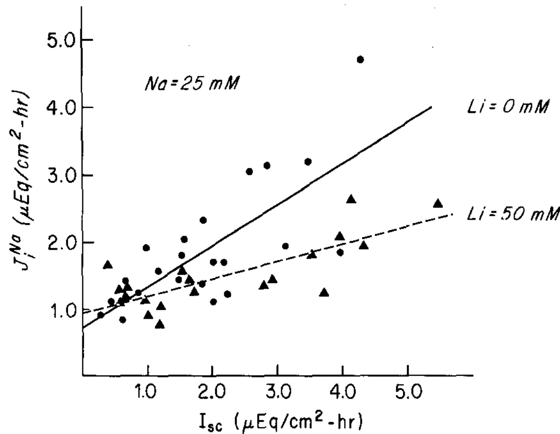

Fig. 7. Plot of J_i^{Na} versus I_{sc} at 25 mM Na in the presence and absence of Li in the mucosal medium

Table 3. Least squares fit for J_i^{Na} vs. I_{sc} at different Li concentrations[a]

[Li] (mM)	Slope	Intercept	r
0	0.61	0.73	0.74
25	0.35	0.89	0.68
50	0.24	0.98	0.76
87	0.17	0.97	0.38

[a] [Na] = 25 mM

25, 50, or 87 mM Li. In each case an equivalent amount of choline – Cl was replaced isosmotically with LiCl.

Due to the great tissue to tissue variability in I_{sc} in the lithium experiments, it is difficult to compare the average values of the Na influxes. The effect of Li on influx is evident, however, in plots of Na influx versus I_{sc}. Fig. 7 shows the linear regression lines for plots of J_i^{Na} versus I_{sc} at 0 mM and 50 mM Li. The intercepts are similar, but the slope is reduced in the presence of Li, suggesting that the effect of Li is primarily on the saturating component of Na influx. Table 3 lists the slopes, intercepts and correlation coefficients for similar plots at different Li concentrations. The intercepts are similar while the slopes decrease with increasing Li concentration, suggesting that the principal effect of Li is to inhibit the saturating component of Na influx.

Some additional insight into the nature of the inhibition of Na influx by Li may be gained by considering a more quantitative comparison of inhibition at different Li concentrations. The previous experiments suggest

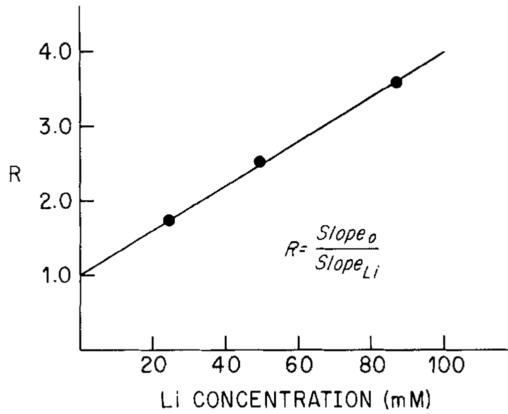

Fig. 8. Plot of the ratio of the slopes relating J_i^{Na} to I_{sc} in the presence and absence of Li. *See text* for details

that at any point on the regression lines shown in Fig. 7, the Na influx is given by the sum of a saturating component, which is correlated with I_{sc}, and a linear component which is not correlated with I_{sc}. The ratio of the slope of the line for [Li] = 0 to the slope of the lines for [Li] > 0 should, therefore, correspond to the ratio of the saturating components of Na influx, i.e.,

$$\frac{[\text{SLOPE}]_{\text{Li}=0}}{[\text{SLOPE}]_{\text{Li}>0}} = \frac{[J_{\text{sat}}^{\text{Na}}]_{\text{Li}=0}}{[J_{\text{sat}}^{\text{Na}}]_{\text{Li}>0}}. \tag{3}$$

Fig. 8 is a plot of this ratio versus the concentration of Li. The least squares line has an intercept not significantly different from 1.0 and a positive slope. As shown by Hajjar and Curran [8] this behavior is characteristic of systems which conform to Michaelis-Menten kinetics and are subject to competitive or noncompetitive inhibition. From the slope of this line, it is possible to calculate a value for the apparent inhibitor constant for Li, K_{Li}. Using the value of 31 mM previously obtained for K_{Na} yields a value for about 18 mM for K_{Li} if the inhibition is competitive.

Discussion

Cofré and Crabbé [5], in their study of the colon of *B. marinus*, found that net Na flux accounted for about 93% of I_{sc} and that I_{sc} was enhanced by aldosterone *in vitro*. The present experiments essentially confirm these results and demonstrate additionally that the observed increase in I_{sc}

caused by aldosterone can be largely attributed to an increase in the simultaneously measured net Na flux. Hogben (*personal communication*) has obtained similar results with the colon of *Rana catesbiana*.

Measurement of the unidirectional influx of Na from the mucosal bathing solution into the mucosal cell layer indicates that Na entry into this tissue can be divided operationally into two components. One component exhibits saturation kinetics, is correlated with short circuit current, and is inhibited by Li in the mucosal solution. The second component varies as a linear function of Na concentration, is not correlated with the short circuit current, and does not appear to be inhibited by Li. Although the mechanisms by which Na enters this tissue have not been clearly defined by these experiments, the data suggest some interesting possibilities.

The linear component of Na influx has some of the characteristics of simple diffusion. The fact that this component of Na entry is not correlated with the short circuit current suggests that it may represent ion movement *via* some pathway not involved in the metabolically linked transport of Na across the tissue. Clearly, it is of interest to determine if the linear component of Na uptake corresponds to Na influx into some "shunt pathway" such as that described by Frizzell and Schultz [7] in the rabbit ileum. A reasonable upper limit for the permeability of the "shunt pathway" in the colon may be obtained from the serosa to mucosa, unidirectional Na flux. In tissues having a total conductance similar to the average conductance in uptake experiments[2] (3.5 mmho/cm^2) the value of the serosa to mucosa Na flux yields an approximate "shunt permeability" of 0.01 cm/hr; only one half that of the linear uptake component. Biber *et al.* [2] noted a similar discrepancy in the linear uptake component of Na influx into frog skin and the Na permeability of the shunt pathway in frog skin measured by Mandel and Curran [10] in transmural flux experiments.

Linear Na influx in excess of that attributable to the shunt could consist of Na uptake into damaged areas of tissue or into cells not involved in net Na transport, or into some other space not accessible to mannitol. Such a discrepancy could also arise if mannitol does not equilibrate with the "extracellular space" during the 30 sec exposure to isotope. Our preliminary experiments on the toad colon, however, confirm ob-

2 The average tissue conductance in the uptake experiments (3.5 mmho/cm^2) was significantly greater than that obtained in transmural flux experiments (2.0 mmho/cm^2). This may suggest that the larger edge-to-surface ratio in the influx chambers produced a greater edge-damage effect around the circumference of these tissues.

servations on the frog skin [2] that the ^{3}H-mannitol space is essentially constant after 30 sec.

The high correlation of the saturable component of Na influx with I_{sc} strongly suggests that this portion of Na entry represents the initial step in the net, active transport of Na by the colon. If Na must enter the cells to be pumped then the characteristics of this component of influx may reflect the properties of the mucosal cell membrane of the epithelial layer. A simple three compartment model for transcellular Na fluxes would require that Na influx into the cellular compartment be equal to or greater than the net Na flux. If the saturable component of Na influx represents the *total* influx into the cells, then the slopes of the plots of J_i^{Na} *vs.* I_{sc} would suggest that the unidirectional influx into the cells is *equal* to the net Na transport across the tissue; i.e., that under short circuit conditions the "back flux" of Na from cell to mucosal solution is vanishingly small. This behavior could indicate the presence of a relatively large (>50 mV, cell negative) electrical potential difference across the mucosal cell membrane under short circuit conditions, or the data may suggest that Na entry occurs by some process other than simple diffusion.

A detailed interpretation of the saturating component of Na entry is complicated by a lack of information concerning the electrical potential difference across the mucosal cell membrane. It is conceivable, for instance, that alterations in the Na concentration or Li concentration in the mucosal medium may alter this potential and thereby alter the Na influx into the cells. Frazier [6], however, found that the electrical potential across the mucosal cell membrane of the urinary bladder of *B. marinus* under short circuit conditions was virtually unchanged when mucosal Na concentration was varied from 114 to less than 1 mm. Similarly, Cereijido and Curran [4] found no significant change in the intracellular potential in frog skin under short circuit conditions when Na concentration in the outside solution was altered. In addition, Biber and Curran [1] reported that in the short circuited state intracellular potentials in frog skin were unaffected by Li in the outside solution. If a similar situation obtains for the toad colon, then the data may suggest that a portion of the Na influx does not proceed by simple diffusion, but rather by some interaction with a component of the mucosal cell membrane. An attractive possibility is that the component of Na influx, which exhibits saturation kinetics and is inhibited by Li, represents the interaction of Na ions with a "site" or "carrier" in the mucosal membrane. Cofré and Crabbé [5], on the basis of the measurements of the apparent "Na pool" in the toad colon, suggested that aldosterone stimulated net Na transport in this tissue by increasing

the permeability of the mucosal cell membrane. Thus, the Na entry step defined by the present experiments may be a possible site of action of aldosterone.

The author is grateful to Drs. C.A.M. Hogben, Q. Al-Awqati, and R.A. Frizzell for their critical reading and discussion of this manuscript and to Dr. T.U.L. Biber for his advice and assistance with experimental technique. I am indebted to Ms. Anne Lamont for able technical assistance. The work was supported by USPHS Grant No. AM-12028 to P.F.C. D.C.D. was the recipient of a USPHS Postdoctoral Fellowship No. 1-R02-AM 5132101.

References

1. Biber, T. U. L., Curran, P. F. 1970. Direct measurement of uptake of sodium at the outer surface of frog skin. *J. Gen. Physiol.* **56**:83
2. Biber, T. U. L., Cruz, L.J., Curran, P. F. 1972. Sodium influx at the outer surface of frog skin. Evaluation of different extracellular markers. *J. Membrane Biol.* **7**:365
3. Bray, G.A. 1960. A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. *Anal. Biochem.* **1**:279
4. Cereijido, M., Curran, P. F. 1965. Intracellular electrical potentials in frog skin. *J. Gen. Physiol.* **48**:543
5. Cofré, G., Crabbé, J. 1967. Active sodium transport by the colon of *Bufo marinus*: Stimulation by aldosterone and antidiuretic hormone. *J. Physiol. (London)* **188**:177
6. Frazier, H.S. 1962. The electrical potential profile of the isolated toad bladder. *J. Gen. Physiol.* **45**:515
7. Frizzell, R.A., Schultz, S.G. 1972. Ionic conductances of extracellular shunt pathway in rabbit ileum. *J. Gen. Physiol.* **59**:318
8. Hajjar, J.J., Curran, P. F. 1970. Characteristics of the amino acid transport system in the mucosal border of the rabbit ileum. *J. Gen. Physiol.* **56**:673
9. Lew, V.L. 1970. Short-circuit current and ionic fluxes in the isolated colonic mucosa of *Bufo arenarum*. *J. Physiol. (London)* **206**:509
10. Mandel, L.J., Curran, P.F. 1972. Response of the frog skin to steady state voltage clamping. I. The shunt pathway. *J. Gen. Physiol.* **59**:503
11. Parsons, D.S., Patterson, C.R. 1965. Fluid and solute transport across rat colonic mucosa. *J. Exp. Physiol.* **50**:220
12. Scatchard, G. 1949. The attractions of proteins for small molecules and ions. *Ann. N.Y. Acad. Sci.* **51**:660
13. Schultz, S.G., Frizzell, R.A., Nellans, H.N. 1974. Ion transport by mammalian small intestine. *Annu. Rev. Physiol.* **36**:51
14. Schultz, S.G., Zalusky, R. 1964. Ion transport in isolated rabbit ileum. I. Short circuit current and Na fluxes. *J. Gen. Physiol.* **47**:567