

Characteristics of Sodium Flux from Serosa to Mucosa in Rabbit Ileum

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ABSTRACT Sodium flux from serosa to mucosa, J_{sm}^{Na} in rabbit ileum in vitro has been studied as a function of applied electrical potential at equal sodium concentrations in the bathing solutions. The results indicate that J_{sm}^{Na} involves two pathways, a diffusional flux through a paracellular shunt pathway and a flux that is independent of applied potential and presumably involves a transcellular pathway. The latter pathway comprises approximately 25% of J_{sm}^{Na} in Ringer's solution containing 10 mM glucose and 25 mM bicarbonate. It is stimulated significantly by theophylline unaffected by removal of glucose or addition of ouabain but is reduced to negligible values by anoxia, dinitrophenol, and replacement of all chloride and bicarbonate by isethionate. Thus this component of J_{sm}^{Na} has a number of characteristics consistent with involvement in a specific secretory process mediating an electrically neutral secretory transport of sodium plus anion from serosa to mucosa. In addition to stimulating this process, theophylline significantly reduced the permeability of the paracellular shunt pathway to sodium.

Recent studies on ion transport across rabbit ileum have raised certain questions about mechanisms involved in movement of ions from the serosal to the mucosal side of the tissue. For example, Frizzell and Schultz (1) have suggested that nearly all of the Na flux from serosa to mucosa across in vitro preparations of rabbit ileum occurs via the intercellular shunt pathway they have described (see also Rose and Schultz [2]). On the other hand, Powell et al. have proposed that there may be an active secretory process for Na in guinea pig ileum (3) and rabbit ileum (4, 5) that accounts for a part of the observed serosal-to-mucosal flux and that this process may be stimulated by cholera toxin (6) and theophylline (5). Such a process might be expected to involve transcellular movement of Na and would not be consistent with purely passive Na movement from serosa to mucosa via a shunt pathway. The situation is further complicated by the suggestion of Field et al. (7, 8) that the

secretory process observed in the presence of cholera toxin or theophylline involves an "electrogenic" anion transport system but perhaps not a specific secretory system for Na. Thus transcellular Na movement would not be required although it could still occur by either active or passive mechanisms. In none of these cases was there entirely compelling evidence for the particular hypothesis. We have, therefore, attempted to consider the problem in a different manner by examining the characteristics of the serosal-to-mucosal Na flux using the approach employed by Frizzell and Schultz (1) to separate the shunt pathway from the (apparent) cellular pathway. In this way, we hoped to determine whether or not there is a "cellular" component of serosal-to-mucosal flux and if there is, to examine some of its characteristics.

METHODS

Theoretical

The net flux of Na, $J_{\text{net}}^{\text{Na}}$, across the ileum is the difference between the unidirectional transmural fluxes from mucosa to serosa, $J_{\text{ms}}^{\text{Na}}$, and from serosa to mucosa $J_{\text{sm}}^{\text{Na}}$;

$$J_{\text{net}}^{\text{Na}} = J_{\text{ms}}^{\text{Na}} - J_{\text{sm}}^{\text{Na}} \quad (1)$$

In certain epithelia such as frog skin (9) and toad bladder (10), $J_{\text{net}}^{\text{Na}}$ has been found equal to the short-circuit current, I_{sc} , the current necessary to reduce the spontaneous potential difference to zero. This equality has not, however, been uniformly observed in preparations of small intestine, (see for example Taylor et al. [11], Powell et al. [3], Binder et al. [4], Field et al. [12], Barry et al. [13]) but has been observed under some conditions (14, 15). Powell et al. (3) suggested that the difference between $J_{\text{net}}^{\text{Na}}$ and I_{sc} observed in guinea pig ileum could be accounted for by the presence of two opposing Na transport systems, a Na-absorbing system that was electrogenic in that it accounted for the short-circuit current and an "electrically silent" or "neutral" secretory system involving transport of NaCl and/or NaHCO_3 from serosa to mucosa. An alternative explanation offered by Field et al. (7, 8) on the basis of their studies on rabbit ileum is that there is an electrogenic transport system for anions that contributes to I_{sc} . In both of these situations, I_{sc} would be greater than $J_{\text{net}}^{\text{Na}}$ as observed experimentally.

In order to examine the possible involvement of a secretory component in $J_{\text{net}}^{\text{Na}}$, we have made use of the approach suggested by Schultz and Zalusky (16) and developed more fully for other purposes by Frizzell and Schultz (1). We assume that the transmural unidirectional flux of Na from serosa to mucosa can, in principle, be described as the sum of a flux through a cellular pathway, $J_{\text{csm}}^{\text{Na}}$, and a diffusional flux through the extracellular shunt, $J_{\text{dsm}}^{\text{Na}}$. Thus

$$J_{\text{sm}}^{\text{Na}} = J_{\text{csm}}^{\text{Na}} + J_{\text{dsm}}^{\text{Na}} \quad (2)$$

We further assume, in accord with the considerations of Frizzell and Schultz (1) that $J_{\text{dsm}}^{\text{Na}}$ can be characterized as a simple diffusion process. On this basis, their analysis indicates that with identical Na concentrations in the two bathing solutions, $J_{\text{dsm}}^{\text{Na}}$

should be simply a function of potential difference (PD). For relatively small values of PD, this relationship can be approximated by the following expression

$$J_{\text{dsm}}^{\text{Na}} = {}_0J_{\text{dsm}}^{\text{Na}} [\exp (F\psi_{\text{ms}}/RT)]^{1/2} = {}_0J_{\text{dsm}}^{\text{Na}} \xi^{1/2}, \quad (3)$$

in which ${}_0J_{\text{dsm}}^{\text{Na}}$ is flux through the shunt pathway under short-circuit conditions, ψ_{ms} is potential difference across the tissue with the mucosal side taken as reference, and F , R , and T have their usual meanings. Thus, Eq. 2 can be written

$$J_{\text{sm}}^{\text{Na}} = J_{\text{osm}}^{\text{Na}} + {}_0J_{\text{dsm}}^{\text{Na}} \xi^{1/2}. \quad (4)$$

In principle, $J_{\text{osm}}^{\text{Na}}$ could also depend on potential difference. However, Frizzell and Schultz (1) have shown that 85–90 % of the current passed across rabbit ileum is carried via the shunt pathway. Thus for reasonable values of external current, there should be little change in $J_{\text{osm}}^{\text{Na}}$ because there is little current flow via the cellular pathway. The present experiments were designed to examine $J_{\text{sm}}^{\text{Na}}$ in terms of Eq. 4 by studying the flux as a function of applied potential (i.e. $\xi^{1/2}$) in order to evaluate $J_{\text{osm}}^{\text{Na}}$.

Experimental

Nonfasted, adult, male, New Zealand rabbits were killed by intravenous injection of 150 mg of Na pentobarbital and the distal ileum was removed rapidly. The piece of intestine, stripped of its serosal and muscle layers as previously described (3) was mounted as a flat sheet between two Lucite half-chambers having an aperture of 1.13 cm². These chambers were connected to jacketed reservoirs containing 10 ml of the bathing solution which was oxygenated, circulated, and maintained at 37°. The bathing solutions were connected via agar bridges (prepared with the appropriate bathing solution) to calomel electrodes for measurement of PD and to Ag-AgCl electrodes for passing current through the tissue. The current required to maintain a particular PD across the tissue was supplied by an automatic clamp voltage which made appropriate correction for the fluid resistance between the PD sensing bridges. The bathing solutions were always identical on both sides of the tissue. Ringer's solution contained (in mM) 140 Na, 5.2 K, 1.2 Ca, 1.2 Mg, 120 Cl, 25 HCO₃, 2.4 HPO₄, and 0.4 H₂PO₄. HCO₃-free solution was made by replacing all HCO₃ with Cl. A Cl-HCO₃-free solution was prepared by replacing Cl and HCO₃ by isethionate. All HCO₃-containing solutions were bubbled with 95 % O₂ + 5 % CO₂ and 100 % O₂ was used for HCO₃-free solutions. When equilibrated with the appropriate gas phase, all solutions had a pH of 7.4. Except when stated, all solutions contained 10 mM glucose. Usually, four pieces of tissue from the same rabbit were studied simultaneously. $J_{\text{sm}}^{\text{Na}}$ was determined using ²²Na as a tracer, but in a few experiments, bidirectional Na fluxes were determined simultaneously on the same piece of tissue using ²²Na and ²⁴Na. Counting techniques have been described previously (3).

Isotopes, and pharmacological agents when used, were added to the bathing solutions immediately after mounting the tissue. Following a 30-min equilibration period under short-circuit conditions, samples for flux determinations were taken every 10 min for 100 min. The transmural PD was clamped at zero from 30 to 50 min, at either

+9 or -9 mV from 50-80 min, at the opposite polarity from 80-110 min, and finally at zero from 110-130 min. The flux determinations under each condition were averaged to give a single value for flux in that tissue at the particular PD. Fluxes changed rapidly in response to changes in PD. Careful inspection of the data indicated that the first 10-min period after a PD change yielded fluxes that did not vary consistently from the other periods at that PD. Consequently, all periods were included in the average. The mean flux values for each PD were then fitted by a straight line according to Eq. 4 by the method of least squares to provide values of J_{osm}^{Na} and ${}_0J_{dsm}^{Na}$ for that tissue. Means and SEM of these values are reported with N equal to the number of tissues. Differences were evaluated by Student's t test. In each experiment, total tissue conductance was determined from PD and applied current; any tissue showing non-constant conductance was discarded.

In order to minimize variations related to the duration of the experiment, the following procedure was used: (a) The same experiment was performed (same bathing solution) on two to four adjacent pieces of ileum from the same rabbit; (b) the voltage clamping was applied in a symmetric way so that when one piece of tissue had a positive PD applied, the adjacent piece had a negative PD, and vice versa; (c) the fluxes in short-circuited conditions were measured at the beginning and the end of each experiment. The transmural PD was varied over the rather narrow range of ± 9 mV in order to minimize the approximation used in the derivation of Eq. 4 (less than 1% over a range ± 10 mV), and possible effects of electroosmotic water flow induced by the applied current (17).

RESULTS

Evidence for a Cellular Flux of Na from Serosa to Mucosa

Unidirectional transmural Na fluxes, J_{sm}^{Na} , were determined at 0, +9, and -9 mV in 37 pieces of tissue from 17 rabbits under control conditions (Ringer solution plus 10 mM glucose). Fig. 1 shows results obtained for five tissues and indicates that J_{sm}^{Na} is a linear function of $\xi^{1/2}$ as predicted by Eq. 4. The slope of each line is taken to represent the diffusional flux through the shunt pathway under short-circuit conditions and the intercept on the y -axis represents Na flux through the cellular pathway.¹ Since the conductance of individual tissues varied by a factor of nearly three and since unidirectional fluxes were approximately proportional to conductance, results for individual tissues were considered and values of ${}_0J_{dsm}^{Na}$ and J_{osm}^{Na} calculated for each tissue. The first question to consider regarding these data is whether or not there is a significant flux through the operationally defined cellular pathway (or in terms of Eq. 4, whether or not the intercepts of the lines such as those shown in Fig. 1 differ significantly from zero on the average). A simple nonparametric test of

¹ The extrapolation to the y -axis is, of course, a mathematical abstraction in that it represents the operation of an infinite driving force, and the system would not be expected to behave linearly over a wide range of PD's. However, the extrapolation provides a convenient way of estimating how much, if any, of the measured fluxes fail to respond to changes in PD over the range examined.

the hypothesis that, on the average, a straight line through the three experimental points passes through the origin is as follows: For each tissue, the line connecting the origin and the point for short-circuit conditions ($\xi^{1/2} = 1$, ${}_0J_{sm}^{Na}$) is drawn. The number of points at -9 mV that lie *above* this line and the number at $+9$ mV that lie *below* the line are noted and compared with the numbers expected for a line through the origin on the basis of the binomial distribution. For a line through the origin, there should be equal numbers of points above and below the line at each PD. At -9 mV the points from 31 of

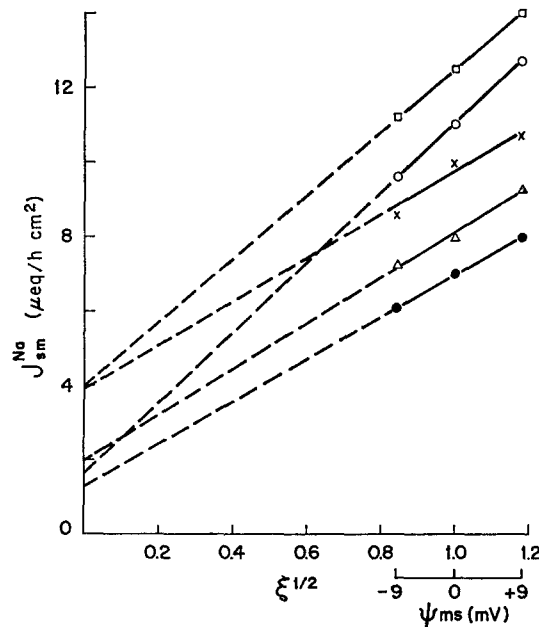


FIGURE 1. Relationship between Na flux from serosa to mucosa, J_{sm}^{Na} , and PD expressed as $\xi^{1/2} = [\exp(F\psi_{ms}/RT)]^{1/2}$ under control conditions for five tissues. The lines were determined by least squares.

37 tissues were above this line and at $+9$ mV the points from 29 of 37 tissues were below the line. These distributions differ significantly ($P < 0.001$) from those expected for a line through the origin. On this basis, the data indicate that $J_{osm}^{Na} > 0$. The mean value of J_{osm}^{Na} for 37 control tissues was 2.38 ± 0.46 (SEM) $\mu\text{eq/h cm}^2$, and negative values of J_{osm}^{Na} were observed in only 4 out of the 37 tissues. This distribution of values of the intercepts also differs significantly from that expected on the hypothesis that the lines for the individual tissues pass through the origin.²

² In addition to the present series of experiments we have carried out for other purposes similar studies on 32 tissues under control conditions. In only two tissues was a negative value of J_{osm}^{Na} observed. Thus in a total of 69 tissues positive values were obtained in 63.

A slightly different approach to analysis of these results is based on the suggestion of Nellans et al. (15). Fluxes for each tissue were normalized to the value obtained under short-circuit conditions. The mean ratio of flux at -9 mV to flux at zero mV was 0.885 ± 0.010 , a value significantly ($P < 0.01$) greater than the value of 0.844 expected if all serosal-to-mucosal flux occurs via the PD-sensitive shunt pathway. The mean ratio at $+9$ mV was 1.138 ± 0.014 , significantly ($P < 0.01$) less than the value of 1.185 expected if all flux occurs via the shunt. A least squares line calculated using these normalized fluxes for individual tissues had a slope of 0.743 ± 0.069 , a value significantly less than unity, and an intercept of 0.262 ± 0.071 , a value significantly greater than zero. Thus under these particular experimental conditions and within the limits of the theory, we conclude that approximately 75% of the serosal-to-mucosal Na flux passes through the shunt pathway and 25% through a parallel, presumably cellular pathway.

Certain aspects of this approach can be evaluated by examining effects of PD on both unidirectional Na fluxes across the tissue and on net flux. According to the above considerations, flux from mucosa to serosa should also involve a cellular and a shunt pathway so that

$$J_{ms}^{Na} = J_{oms}^{Na} + {}_0J_{dms}^{Na} \xi^{-1/2}. \quad (5)$$

If movement via the shunt pathway is due to simple diffusion, ${}_0J_{dms}^{Na} = {}_0J_{dsm}^{Na}$ and under short-circuit conditions, ($\xi = 1$), the net Na flux should be given by

$${}_0J_{net}^{Na} = J_{oms}^{Na} - J_{osm}^{Na}. \quad (6)$$

To test these points, three experiments were carried out in which J_{ms}^{Na} and J_{sm}^{Na} were measured simultaneously as functions of PD. The results are summarized in Fig. 2. The slopes of the two lines do not differ significantly and ${}_0J_{dms}^{Na}/{}_0J_{dsm}^{Na} = 0.96$. The difference of the intercepts on the y axis, which should represent $J_{oms}^{Na} - J_{osm}^{Na}$, is $3.19 \mu\text{eq/h cm}^2$ almost exactly equal to the measured net Na flux under short-circuit conditions, $3.11 \mu\text{eq/h cm}^2$.

These experiments also permit us to examine another point. If Na movement through the shunt is due to simple diffusion and therefore driven entirely by concentration and electrical gradients,

$$J_{dnet}^{Na} = P_{Na} C_{Na} \frac{[F\psi_{ms}]}{RT}, \quad (7)$$

under conditions of uniform concentration. In Eq. 7, P_{Na} is Na permeability of the shunt and C_{Na} is Na concentration. Thus in the presence of a PD, Eq. 6 should take the form

$$J_{net}^{Na} = J_{oms}^{Na} - J_{osm}^{Na} - \frac{P_{Na} C_{Na} F\psi_{ms}}{RT} \quad (8)$$

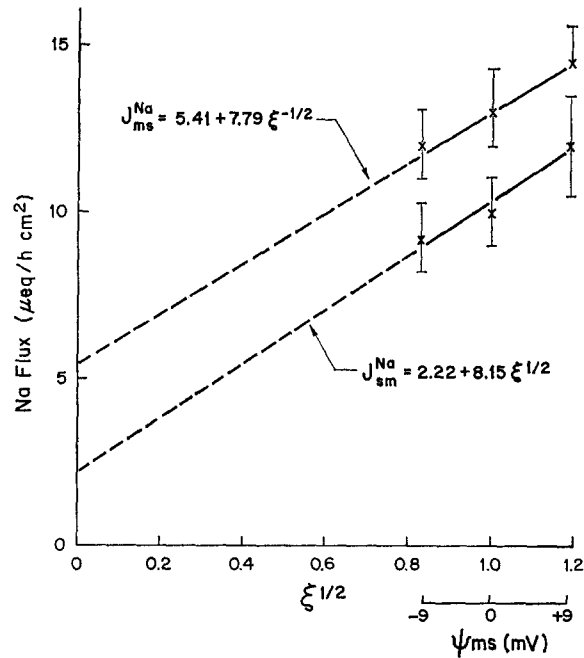


FIGURE 2. Relationship between unidirectional Na fluxes and PD. The points are average values (\pm SEM) from three experiments in which the two fluxes were measured simultaneously. Note that the sign of the exponent $\frac{1}{2}$ is different for fluxes in the two directions. The lines were determined by least squares and their equations are given.

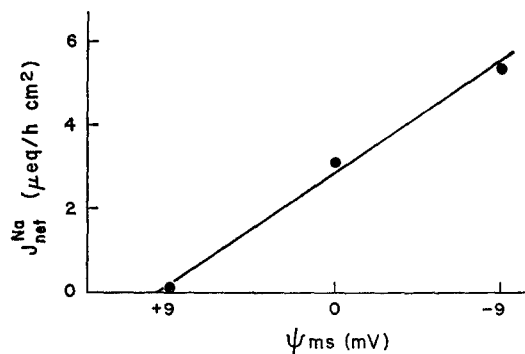


FIGURE 3. Relationship between net Na flux and transmembrane PD determined in three experiments in which bidirectional Na fluxes were measured simultaneously.

As shown in Fig. 3, net Na flux in these experiments was a linear function of ψ_{ms} as expected from Eq. 8 and the quantitative relationship is given by

$$J_{net}^{Na} = -0.30 \psi_{ms} + 2.83. \quad (9)$$

Similar relationships were obtained by Schultz and Zalusky (16) for rabbit ileum and intact muscle (slope = -0.32 , intercept = 3.56) and by Clarkson (17) for rat ileum (slope = -0.13 , intercept = 2.01). It is of interest to note that in our preparation of rabbit ileum under these experimental conditions, a PD of $+9$ mV (serosa positive) is sufficient to reduce net Na flux to zero.

Properties of J_{osm}^{Na}

In an effort to obtain further information regarding the nature of the cellular component of serosal-to-mucosal Na flux, we have examined the effects on it of a variety of agents and of composition of the bathing solutions. The results of some of these experiments are summarized in Table I in terms of short-

TABLE I
PROPERTIES OF Na FLUX FROM SEROSA TO MUCOSA

	${}_0J_{sm}^{Na}$	J_{osm}^{Na}	${}_0J_{dsm}^{Na}$	I_{sc}	G_T	P_{Na}
			$\mu\text{eq/h cm}^2$		mmho/cm^2	cm/h
Control (37, 17)	9.62	2.43	7.13	3.24	20.2	0.051
	± 0.37	± 0.34	± 0.48	± 0.26	± 0.8	± 0.004
No glucose (8, 2)	8.25	2.94	5.24	1.44*	17.9	0.036
	± 0.66	± 0.76	± 0.67	± 0.10	± 1.3	$\pm .005$
DNP (15, 5)	9.48	0.46*	8.98‡	0.10*	20.6	0.064‡
	± 0.65	± 0.70	± 0.81	± 0.07	± 1.6	± 0.006
Anoxia (13, 4)	16.18*	-0.58^*	16.85*	-0.05^*	21.3	0.110*
	± 3.30	± 0.89	± 3.86	± 0.01	± 2.4	± 0.027
Ouabain (16, 5)	9.60	2.84	6.64	0.27*	16.3*	0.046
	± 0.42	± 0.60	± 0.59	± 0.06	± 0.7	± 0.004
Theophylline (21, 7)	9.77	4.45*	5.30‡	3.79	17.1*	0.038‡
	± 0.61	± 0.58	± 0.66	± 0.88	± 1.1	± 0.005

Numbers in parentheses give number of tissues followed by number of animals.

${}_0J_{sm}^{Na}$ is the observed flux under short-circuit conditions while J_{osm}^{Na} and ${}_0J_{dsm}^{Na}$ are calculated as described under Methods (see Eq. 4).

$$P_{Na} = {}_0J_{dsm}^{Na}/[Na]_s$$

* Significantly different from control, $P < 0.01$

‡ Significantly different from control, $P < 0.05$

circuit current, I_{sc} , serosal-to-mucosal Na flux under short circuit conditions, J_{osm}^{Na} and ${}_0J_{dsm}^{Na}$. Total tissue conductance, G_T , and the permeability coefficient of the shunt pathway, P_{Na} ($= {}_0J_{dsm}^{Na}/[Na]_s$) are also given. The values reported are averages for individual tissues. Removal of glucose from the bathing solutions caused a decrease in I_{sc} but no significant change in total serosal-to-mucosal flux at short circuit as observed previously by Schultz and Zalusky (16) for rabbit ileum with intact muscle layers. The cellular component of Na flux was not significantly altered by glucose removal; there appeared to be a decrease in Na flux via the shunt pathway but the change was not statistically significant ($P \approx 0.075$). These results indicate that exogenous substrate is not

essential for $J_{\text{osm}}^{\text{Na}}$ and might be interpreted as an indication that this flux component is not dependent on metabolism. However, in this regard it is of interest to note that Frizzell et al. (18) have found that addition of glucose to the bathing solution does not increase oxygen consumption of rabbit ileum, suggesting that endogenous substrates are sufficient to maintain adequate metabolism. Thus lack of an effect of glucose on $J_{\text{osm}}^{\text{Na}}$ does not necessarily indicate that the flux is independent of metabolism.

To examine this point more explicitly, we have carried out experiments involving anoxia and treatment of the tissue with 2,4-dinitrophenol (DNP). Results are summarized in rows 3 and 4 of Table I. DNP decreased I_{sc} to zero but had no significant effect on ${}_0J_{\text{sm}}^{\text{Na}}$. There was an increase in flux through the shunt pathway, ${}_0J_{\text{dsm}}^{\text{Na}}$, and $J_{\text{osm}}^{\text{Na}}$ was reduced significantly below the control value and to a level that did not differ significantly from zero. Although experiments in the presence of N_2 were not entirely satisfactory because of considerable scatter of the data, the results are similar to those obtained with DNP in that $J_{\text{osm}}^{\text{Na}}$ was reduced to zero. Thus the results of these two sets of experiments indicate that the cellular component of serosal-to-mucosal Na flux depends strongly on metabolism; it could therefore involve an active process.

In view of the suggestion of Powell et al. (3) that the Na secretory process in guinea pig ileum was not markedly affected by ouabain, we have also examined the effect of this agent. As indicated in Table I, ouabain (10^{-4}M) decreased I_{sc} nearly to zero but had no effect on $J_{\text{osm}}^{\text{Na}}$ or on ${}_0J_{\text{sm}}^{\text{Na}}$. Tissue conductance decreased significantly below control levels but the decrease in ${}_0J_{\text{dsm}}^{\text{Na}}$ was not statistically significant. Finally, in view of the observation that theophylline stimulates ionic secretory processes in intestine (7, 18, 5), the effect of this agent was studied with results shown in the last row of Table I. Theophylline (10^{-2}M) increased $J_{\text{osm}}^{\text{Na}}$ significantly above the control level ($P < 0.01$) and significantly decreased both tissue conductance and ${}_0J_{\text{dsm}}^{\text{Na}}$. The difference between controls and theophylline-treated tissues is shown in Fig. 4 in terms of average values of fluxes normalized to flux under short-circuit conditions as functions of $\xi^{1/2}$. Although the differences seem small the normalized flux at -9 mV is significantly greater in theophylline-treated tissues than in controls and the reverse is true at $+9\text{ mV}$. Thus, the slope of the line (${}_0J_{\text{dsm}}$) is decreased by theophylline and the intercept (J_{osm}) is increased.

The effects of alterations in the anion composition of the bathing solutions are summarized in Table II. Removal of all HCO_3 from the solution led to a significant decrease in $J_{\text{osm}}^{\text{Na}}$ ($P < 0.05$). Replacement of Cl and HCO_3 in the bathing solution yielded a value of $J_{\text{osm}}^{\text{Na}}$ that was not significantly different from zero. This treatment also led to a substantial increase in tissue conductance and flux through the shunt pathway. Under these conditions, experiments were less satisfactory as indicated by the fact that the calculated value of $J_{\text{osm}}^{\text{Na}}$ was negative, a situation that is physically impossible, but that can

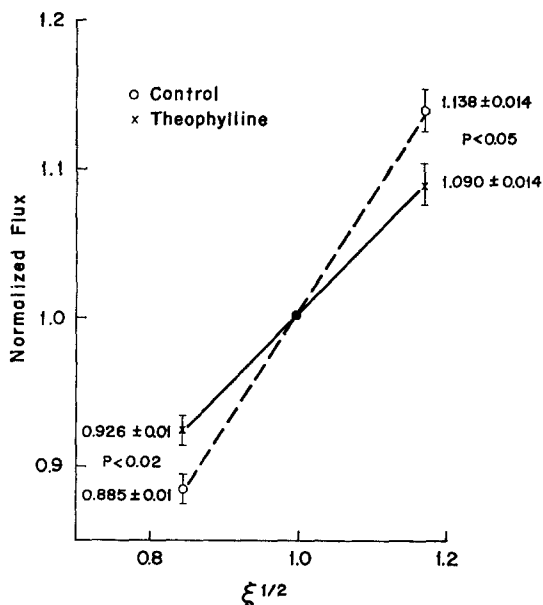


FIGURE 4

FIGURE 4. Relationship between fluxes normalized to flux under short-circuit conditions and $\xi^{1/2}$. Points are average values from 37 control tissues and 20 tissues treated with theophylline.

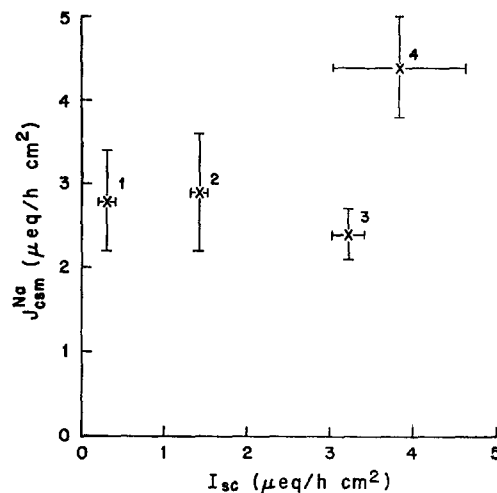


FIGURE 5

FIGURE 5. Relationship between short-circuit current, I_{sc} , and Na flux via the cellular pathway. Points are average values \pm SEM for 1, ouabain; 2, no glucose; 3, control; 4, theophylline.

TABLE II
EFFECT OF SOLUTION COMPOSITION ON Na FLUX FROM SEROSA TO MUCOSA

	ωJ_{sm}^{Na}	J_{osm}^{Na}	ωJ_{dsm}^{Na}	I_{sc}	G_T	P_{Na}
		$\mu\text{eq/h cm}^2$			mmho/cm^2	cm/h
Control (37, 17)	9.62 ± 0.37	2.43 ± 0.34	7.13 ± 0.48	3.24 ± 0.26	20.2 ± 0.8	0.051 ± 0.004
HCO ₃ -free (19, 5)	8.23 ± 0.96	1.15* ± 0.53	7.49 ± 0.99	2.47 ± 0.50	20.0 ± 2.4	0.054 ± 0.007
Cl-HCO ₃ -free (12, 4)	12.01‡ ± 0.77	-0.94‡ ± 0.59	12.97‡ ± 0.93	1.84‡ ± 0.36	29.4‡ ± 3.0	0.092‡ ± 0.007

* Significantly different from control, $P < 0.05$.

‡ Significantly different from control, $P < 0.01$.

easily arise because of small errors in the rather high measured fluxes. Nonetheless, it appears that J_{osm}^{Na} must be quite small in the absence of Cl and HCO₃ in the bathing solutions.

The above results are consistent with the concept, proposed by Powell et al. (3) that the secretory process for Na is "neutral" or "electrically silent" in that it appears to require the presence of appropriate anions. If this is correct, J_{osm}^{Na} should be independent of I_{sc} . As shown in Fig. 5, there is no consistent rela-

tionship between these two quantities for control experiments and those in the absence of glucose and in the presence of ouabain and theophylline. DNP and N_2 and Cl-HCO_3 free solution all reduce both I_{sc} and J_{osm}^{Na} but the results in Fig. 5 clearly suggest that there is not a necessary connection between I_{sc} and J_{osm}^{Na} .

DISCUSSION

Transcellular Na Movement

Schultz and Zalusky (16) and Clarkson (17) have developed the concept of two parallel pathways for Na movement across the intestinal mucosa, one involved in active transport and one permitting only passive Na movement. The situation has been made more complex recently by suggestions that the "active" pathway itself may involve two presumably parallel routes for Na transport, one involved in Na absorption and one involved in Na secretion (3, 4). One of the major purposes of the work reported here was to examine this possibility by asking whether there was any Na movement from serosa to mucosa that could not be accounted for by the passive pathway. The studies of Rose and Schultz (2) and Frizzell and Schultz (1) on rabbit ileum have indicated quite clearly that the major route of passive Na movement across the mucosa is via a paracellular shunt pathway. This pathway has a much lower resistance than the transcellular pathway so that 85–90% of the electric current passed across the mucosa bypasses the cells. This observation provides the basis of the method used here since passage of current across the tissue should alter Na flux through the shunt pathway significantly but should cause little change in Na movement through the high resistance cellular pathway.

The observation that in Ringer's solution (containing 25 mM HCO_3) approximately 25% of the serosal-to-mucosal Na flux under short-circuit conditions is independent of PD (Fig. 1 and Table I) is thus consistent with the presence of a significant transcellular pathway for Na movement in this direction. This result does not seem to agree with the one reported earlier by Schultz and Zalusky (16). Using a similar technique, they found that virtually all Na movement from serosa to mucosa across rabbit ileum with intact muscle layer could be accounted for by flux through a passive, potential-sensitive pathway. It is, therefore, appropriate to consider possible differences between these two studies. Schultz and Zalusky used a solution containing only 2.5 mM HCO_3 , a condition that in our hands appears to lead to a substantial reduction in the transcellular component of J_{sm}^{Na} (Table II) and would make this component more difficult to detect. In addition, the difference between their observations and ours may be less apparent than appears at first sight. If fluxes measured at PD's of +50 and -30 mV are eliminated, their data (Fig. 6 of reference 15) can be fitted by a line with an intercept (J_{osm}^{Na}) of approximately 1.2 $\mu\text{eq/h cm}^2$, a value that agrees rather well with the one we have obtained

under comparable conditions. Exclusion of the data at the high PD's is not unreasonable since the approximation $\xi^{1/2}$ (Eq. 3) is less accurate here and there may also be significant changes in tissue properties under these conditions.

Nellans et al. (15) have recently examined this point further using rabbit ileum stripped of muscle layers and bathed in a solution containing 10 mM HCO_3 and 12.6 mM K at a pH of 7.1–7.2. They found a linear relationship between J_{sm}^{Na} and $\xi^{1/2}$ with an intercept at the origin (i.e. $J_{osm}^{\text{Na}} = 0$). As noted by Nellans et al., there are several differences between their conditions and our control conditions that might reduce J_{osm}^{Na} including lower HCO_3 and higher K concentrations. They also noted that the technique we have used to correct for fluid resistance between the PD sensing bridges when passing current across the tissue may lead to an overcorrection so that the imposed PD's are different than expected. They have corrected their data for this effect. We have not made such corrections, but it is important to note that the correction will *decrease* the slope of the line such as that shown in Fig. 1 and *increase* the intercept. Thus the difference between their results and ours cannot be explained by this factor.

Two other factors may be involved in the differences between these two studies. First, Nellans et al. used a much wider range of PD's (–40 to +45 mV) than we have. Second, their tissues appear to have an average resistance of approximately 30 Ωcm^2 while our tissues had an average resistance of 50 Ωcm^2 . Under one condition, Cl- HCO_3 -free solution, we observed a tissue resistance of 33 Ωcm^2 and found on the average, a negative value of J_{osm}^{Na} . As discussed by Nellans et al. this represents a physically impossible situation. The result suggests, however, that this type of experiment is difficult to carry out accurately when tissue resistance is extremely low even if a narrow range of imposed PD's is employed. In the final analysis, we have no convincing explanation for the difference between these two sets of experiments. However, we feel confident that in our population of rabbits under control conditions there is a component of serosal-to-mucosal Na flux that does not respond to PD and presumably represents transcellular transfer as opposed to movement through a shunt pathway. For other purposes, we have carried out additional similar experiments in Ringer solution (32 tissues) with results virtually identical to those shown in Table II.

The fact that the present technique suggests that there is a transcellular component to J_{sm}^{Na} does not, by itself, provide any information about the nature of this flux. It could, for example, be entirely a passive flux that does not appear to respond to PD because the PD across the cellular membranes is little altered under our experimental conditions. However, the results presented in Table I indicate that J_{osm}^{Na} is dependent on metabolism and make it unlikely that this dependence can be explained simply in terms of alterations of intracellular ionic concentrations or membrane potentials due to inhibition of

metabolism. Thus $J_{\text{osm}}^{\text{Na}}$ is markedly inhibited by DNP and by anoxia but is unaffected by ouabain although all three treatments should cause relatively similar changes in ionic composition of the cells. These results suggest, but certainly do not prove, that $J_{\text{osm}}^{\text{Na}}$ may be at least in part due to an active transport process but one that does not appear to involve a Na-K-ATPase since it is not inhibited by ouabain. Such a transport system would be consistent with the suggestions of the presence in guinea pig (3) and rabbit ileum (4, 5) of a secretory system for Na transport that, in the guinea pig at least, is not ouabain sensitive.

In addition, Powell et al. (3) have proposed that this secretory system transports NaCl and/or NaHCO₃ and is therefore neutral or electrically silent in guinea pig and have suggested that a similar system is present in rabbit ileum. Several aspects of the present results are consistent with this concept. As shown in Fig. 4, there is no relationship between $J_{\text{osm}}^{\text{Na}}$ and I_{sc} . As indicated in Table II, removal of HCO₃ from the bathing solutions decreases $J_{\text{osm}}^{\text{Na}}$ significantly and removal of both Cl and HCO₃ reduces $J_{\text{osm}}^{\text{Na}}$ to zero. Finally, $J_{\text{osm}}^{\text{Na}}$ is increased by theophylline an agent that leads to net secretion of Na (5) and Cl (7, 15, 5) in rabbit ileum, presumably as a result of increased levels of cyclic AMP in the cells (15, 19). Such an effect might for example be related to the phenomenon observed in toad bladder where increased C-AMP leads to an increased Na permeability of the mucosal membrane (20). If a similar effect occurred in intestine, it should result in an increase in the rate of tracer movement from serosa to mucosa via the transcellular pathway.

Although these results appear to be in agreement with several of our previous suggestions regarding a specific Na secretory process, they provide no information on the location of this cellular component of Na flux. It could reside in the villous cells, in the crypt cells or in any region of the tissue in which changes in transmural PD yield minimal changes in the local PD. Interpretation of the behavior of this component of serosal-to-mucosal Na flux is rather strongly dependent on the specific pathway involved. If the flux occurs mainly through the villous cells that are thought to be responsible for ion and water absorption, the relationship of this flux to the primary active step in Na absorption at the lateral/basal membrane and to the theophylline-sensitive, coupled NaCl influx at the brush border described by Nellans et al. (21) must be considered. If the flux $J_{\text{osm}}^{\text{Na}}$ involves primarily a separate pathway parallel to the absorptive route, there need be no clear-cut relationship between the two pathways and interpretations might be quite different. Since we have no information as to the routes involved, extensive speculation about mechanisms of various effects seems unwarranted at present. However, it is perhaps worthwhile to consider one or two aspects of the problem. The model proposed by Nellans et al. (21) for the coupled NaCl influx across the brush border suggests that it should be reversible and hence capable of mediating

NaCl efflux from cell to mucosal solution. It could, therefore, be involved in $J_{\text{osm}}^{\text{Na}}$. If this were true, it would be necessary to explain why $J_{\text{osm}}^{\text{Na}}$ is stimulated by theophylline (Table I) while the coupled NaCl influx is inhibited by this agent (21). According to the model of the process such behavior would not be impossible; a given agent could have different effects on influx and efflux via the proposed transport system and Nellans et al. (21) have suggested that this occurs for the coupled process. Another puzzling observation would seem to be the recent finding of Powell et al. (5) that the net Na secretion in rabbit ileum stimulated by theophylline is inhibited by ouabain. This result, which is different than that reported for guinea pig ileum (3), does not appear to be consistent with our observation that $J_{\text{osm}}^{\text{Na}}$ is unaffected by ouabain. Again explanations are possible but are dependent on the nature of the pathway involved in $J_{\text{osm}}^{\text{Na}}$ as we have determined it. For example, if $J_{\text{osm}}^{\text{Na}}$ involved the villous cells, one could argue that ouabain might cause an increase in tracer flux from serosa to mucosa as a result of inhibition of Na extrusion at the serosal side and a rise in cellular Na concentration. In this case, the fact that we observe no rise in $J_{\text{osm}}^{\text{Na}}$ with ouabain could be used to argue that this agent actually inhibits some step in the process.

These points illustrate some of the difficulties in attempting to interpret our results specifically in terms of mechanism. At present, we can only say that there is an apparent cellular component to serosal-to-mucosal Na flux. The finding of such a component is essential if an active secretory system for Na exists in rabbit ileum but does not by itself prove the existence of such a system. Conversely, given the known properties of rabbit ileum, failure to find a value of $J_{\text{osm}}^{\text{Na}}$ greater than zero would have provided a strong argument against active Na secretion. However, although several of the present observations seem consistent with prior suggestions regarding the postulated secretory system, specific interpretations regarding possible mechanisms involved must await further study.

Na Movement Through the Shunt Pathway ${}_0J_{\text{dsm}}^{\text{Na}}$

The fundamental assumption used in this approach to determining ${}_0J_{\text{dsm}}^{\text{Na}}$ is that the Ussing flux ratio equation can be applied to passive ion movements through the shunt path. The data summarized in Fig. 2 provide some direct justification for this assumption. In these experiments, Na movements through the shunt path in both directions were determined simultaneously on a single piece of tissue. The ratio ${}_0J_{\text{dms}}^{\text{Na}}/{}_0J_{\text{dsm}}^{\text{Na}}$ was found to be 0.96 compared to the expected value of unity for zero PD and identical Na concentrations in the bathing solutions.

The results obtained in this study are in agreement with several earlier observations and also provide some additional insight into certain properties of the shunt pathway. The data in Fig. 2 can be used to provide a relationship

between net Na flux and current that has the form

$$J_{\text{net}}^{\text{Na}} = -0.39 I + 3.81, \quad (10)$$

in which both $J_{\text{net}}^{\text{Na}}$ and I are expressed as $\mu\text{eq}/\text{h cm}^2$. Since Na concentration is identical in the two bathing solutions, the coefficient multiplying I in Eq. 10 is the transference number, t_{Na} , for Na in the shunt pathway. The observed value is identical to t_{Na} in 140 mM NaCl at 25°C (0.38). Clarkson (17) observed a similar relationship between Na flux and current in rat ileum with a slightly higher value of t_{Na} of 0.51. A second estimate of t_{Na} for the shunt pathway may be obtained from the ratio of partial Na conductance (numerically equal to ${}_0J_{\text{dsm}}^{\text{Na}}$ [22]) to total tissue conductance. For control conditions, the data in Table I yield a value of 0.35 ($= 7.13/20.2$), a value that agrees well with the finding of Schultz et al. (23) and Nellans et al. (15) that removal of all Na from the solutions bathing rabbit ileum leads to a 40% decrease in tissue conductance. However, this value should be somewhat of an underestimate of t_{Na} since the conductance of the shunt pathway is smaller than total tissue conductance. A more appropriate estimate can be obtained by plotting ${}_0J_{\text{dsm}}^{\text{Na}}$ against tissue conductance as shown in Fig. 6. Within the range of conductances observed, the relationship is approximately linear suggesting that the permeability coefficient for Na does not vary significantly with conductance.

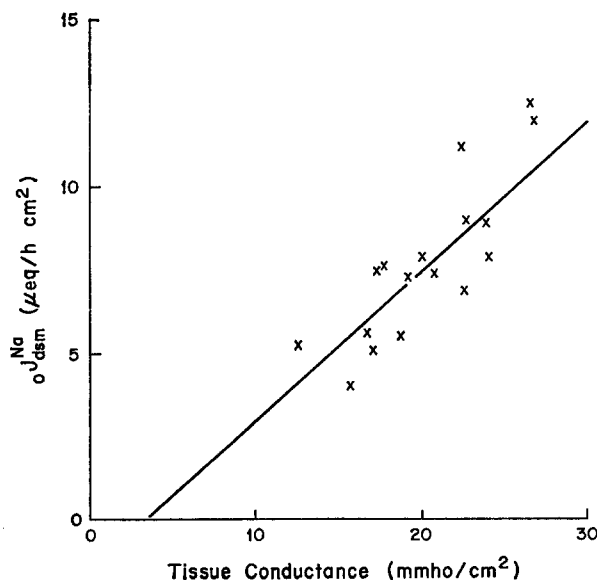


FIGURE 6. Relationship between Na flux from serosa to mucosa through the shunt pathway under short-circuit conditions, ${}_0J_{\text{dsm}}^{\text{Na}}$, and total tissue conductance. Each point represents the average value obtained from tissues taken from a single rabbit. The line was determined by least squares.

The least squares line is given by

$${}_0J_{\text{dsm}}^{\text{Na}} = 0.45 G_T - 1.51,$$

in which G_T is total tissue conductance. Since t_{Na} in the shunt is given by G_s^{Na}/G_s in which G_s^{Na} is partial Na conductance and G_s is total shunt conductance,

$$G_s^{\text{Na}} = t_{\text{Na}}G_s,$$

and since total tissue conductance, G_T is the sum of G_s and conductance of the cellular pathway, G_c ,

$$G_s^{\text{Na}} = t_{\text{Na}}G_T - t_{\text{Na}}G_c.$$

Since ${}_0J_{\text{dsm}}^{\text{Na}}$ expressed in $\mu\text{eq/h cm}^2$ is numerically equal to G_s^{Na} , the slope of the line in Fig. 6 is equal to t_{Na} and the intercept on the x -axis should represent the conductance of the cellular pathway. This value of G_c , 3.4 mmho/cm² is 16% of the average total tissue conductance 20.2 mmho/cm². This result is in reasonable agreement with the estimate of Frizzell and Schultz (1) that 85–90% of total tissue conductance of rabbit ileum resides in the shunt pathway.

Frizzell and Schultz (1) have also characterized Na movement in the shunt pathway of rabbit ileum by measuring the effects of PD on unidirectional Na flux from the mucosal solution into the tissue. Thus they determined Na influx into the shunt as opposed to movement across the entire shunt as determined in the present experiments. In most respects, the results of these two studies are quite similar. In the presence of 140 mM Na but without glucose, they observed an influx into the shunt of 4.9 $\mu\text{eq/h cm}^2$, and we have found a value of 5.2 $\mu\text{eq/h cm}^2$ for flux through the shunt under similar conditions. The agreement is excellent in spite of the fact that Frizzell and Schultz used tissue with intact muscle while we removed most of the muscle layers. The major difference in the two studies appears to lie in estimates of t_{Na} which is given as 0.60 by Frizzell and Schultz (1) compared to our value of 0.45. However, this estimate depends strongly on values of conductance which differ by a factor of 2 for the two preparations and further studies will be necessary to clarify the point and to evaluate further the ionic selectivity of the shunt pathway.

Several of the conditions examined appear to alter properties of the shunt pathway. Theophylline caused a significant decrease in both ${}_0J_{\text{dsm}}^{\text{Na}}$ and total tissue conductance and removal of glucose appeared to cause similar changes but with the limited number of experiments performed we were unable to demonstrate that the latter changes were significant. On the other hand, anoxia and Cl-HCO₃-free solution caused increases in both ${}_0J_{\text{dsm}}^{\text{Na}}$ and tissue

conductance, while DNP increased ${}_0J_{\text{dsm}}^{\text{Na}}$ with no change in conductance and ouabain decreased conductance with no change in ${}_0J_{\text{dsm}}^{\text{Na}}$. Such changes are probably associated with changes in the dimensions of the lateral space between adjacent epithelial cells (24) and perhaps with the rate of fluid transport via these spaces. In this regard, it is perhaps of interest to consider the possible relative roles of the "tight" junction itself and the lateral space in controlling permeability of the paracellular shunt pathway. For example, the calculations of Smulders et al. (24) have indicated that under "normal" conditions in gall bladder, the spaces make insignificant contributions to conductance and sucrose permeability, but that if the spaces are collapsed by making the mucosal solution hypertonic, their contribution becomes significant compared to the junction. Since the junction and space are in series, the overall permeability of the complex should be given by

$$\frac{1}{P_s} = \frac{1}{P_{\text{TJ}}} + \frac{1}{P_{\text{LS}}},$$

in which P is permeability and the subscripts S, TJ, and LS refer to shunt, tight junction, and lateral space, respectively. An attempt to estimate P_{LS} can be made as outlined by Smulders et al. (24) from the relation

$$P_{\text{LS}} = DA/l,$$

in which D is the diffusion coefficient of Na (taken as the free solution value), A is the area of the lateral spaces per unit serosal area, and l is length of the spaces. Detailed microscopic studies of rabbit ileum required to estimate A and l in this equation do not seem to be available. As indicated by Smulders et al. (24) the parameters required are effective height of the cells, width of the intercellular space, and cellular circumference per unit serosal area. The mucosal area per unit serosal area is also required.

The best data on these quantities appear to exist for human intestine. Marsh and Swift (25) give mean values of 7.6×10^{-4} cm for cell diameter and 45×10^{-8} cm² for mucosal surface area. According to Trier (26, 27), the mucosal area per unit serosal area is approximately 8, and the width of the intercellular spaces is in the range of 100–250 Å. (The spaces in intestine are quite narrow through most of the length of the cells (26, 27) and according to Jackson and Cassidy (28) do not show marked variation in width with rate of fluid transport. However, Tomasini and Dobbins (29) did observe dilation of the spaces in the presence of fluid transport, particularly along the basal half of the cells.) These figures lead to a calculated value of A ranging from 0.025–0.063 cm²/cm² of serosal area. The length of the cells in rabbit ileum is approximately 20×10^{-4} cm and since some interdigitation of adjacent cells occurs (26) a reasonable estimate of l might be 30×10^{-4} cm. Introducing these values,

together with the free solution diffusion coefficient for Na (1.1×10^{-5} cm²/s) yields P_{LS} ranging from 9.2×10^{-5} (for $A = 0.025$) to 23×10^{-5} cm/s (for $A = 0.0625$). We have found $P_{Na} = 1.4 \times 10^{-5}$ cm/s under control conditions and 1.0×10^{-5} cm/s in the absence of glucose. Thus according to these estimates the tight junction contributes a minimum of 85–90% of the resistance of the shunt pathway to Na. This conclusion is in agreement with the fact that we have found that flux through the entire shunt is essentially the same as influx into the shunt determined by Frizzell and Schultz (1) (which should depend mainly on P_{TJ}). However, if P_{LS} is less than an order of magnitude greater than P_{TJ} , the permeability of the shunt could be significantly influenced by relatively small changes in dimensions of the lateral spaces. Such behavior would, for example, be consistent with the observations of Esposito et al. (30) that the permeability of rat jejunum to acetamide and thiourea is decreased significantly if fluid absorption is inhibited by lowering the Na concentration of the bathing solutions. Thus inhibition of salt and water absorption caused by ouabain or no glucose could lead to collapse of spaces and decreased conductance of the paracellular pathway. However, the exact consequences of a particular treatment will depend on relative changes in conductance of the cellular and paracellular pathways and on possible changes in t_{Na} in the shunt pathway (31). We do not have sufficient data at present to provide unequivocal explanations for the observed changes in behavior of the shunt pathway and further experiments directed explicitly at this problem are essential.

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