J. Membrane Biol. 31, 189–208 (1977)

Na and Cl Transport and Short-circuit Current in Rabbit Ileum

Yuan-Heng Tai*, Jehan-F. Desjeux, Giustina Danisi, and Peter F. Curran**

Department of Physiology, Yale University School of Medicine, New Haven, Connecticut 06510, and Department of Pediatrics, Universite Paris VII, Hopital Herold, Paris, France

Received 20 November 1975; revised 5 April 1976; revised again 29 July 1976

Summary. Na and Cl fluxes and short-circuit current (I_{sc}) in rabbit ileum have been studied as a function of ionic concentrations in HCO_3-free solutions. Both net Na flux (J_{net}^{Na}) and I_{sc} show similar saturation functions of [Na] at fixed [Cl]. They show no significant difference between zero and 112 mM Na but at 140 mM Na I_{sc} is significantly greater than the J_{net}^{Na}. Net Cl transport, secretion, is observed only at 140 mM Na and is approximately equivalent to the difference between the I_{sc} and J_{net}^{Na}. The transcellular mucosa-to-serosa Na fluxes measured at 140 and 70 mM Na do not differ significantly from the corresponding I_{sc}. The net Cl flux varies with [Cl] at fixed [Na] while I_{sc} is virtually not affected by [Cl]. These results suggest that the absorptive Na transport process is electrogenic and responsible for the I_{sc} and that the secretory fluxes of Na and Cl are coupled, require high [Na], vary with [Cl], and do not contribute to I_{sc}. K-free solution abolishes the I_{sc} after a prolonged lag. Finally, the effect of a low resistance shunt pathway on active Na absorption is examined with a four-compartment model.

Recent studies on ion transport across rabbit ileum have raised questions about the nature of the Na and Cl transport and the relationship of this transport to the short-circuit current, i.e., the current necessary to maintain a zero membrane potential between two identical solutions perfused on the both sides of the tissue. In certain epithelia, such as frog skin [30] and toad bladder [18], the net Na flux, J_{net}^{Na}, has been found to be equal to the short-circuit current, I_{sc}. This equality has not, however, been observed in all preparations of small intestine [1, 3, 12, 28]. Powell, Binder, and Curran [20] and Binder, Powell, Tai, and Curran [3] have suggested that the difference between the J_{net}^{Na} and I_{sc} observed in guinea

* *Present address:* Department of Gastroenterology, Division of Medicine, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D.C. 20012 (to whom reprint requests should be addressed).

** Deceased (October 16, 1974).

pig and rabbit ileum could be accounted for by the presence of two opposing Na transport systems, an electrogenic Na-absorbing system which accounts for the I_{sc} and an "electrically neutral" secretory system involving transport of NaCl and/or $NaHCO_3$ from serosa to mucosa. An alternative explanation offered by Field, Fromm, and McColl [12], Field [10], and Field, Fromm, Al-Awqati, and Greenough [11] on the basis of their studies on rabbit ileum is that there is an electrogenic transport system for anions that also contributes to I_{sc}. In both of these situations, I_{sc} would be greater than the J_{net}^{Na} observed experimentally.

In order to further examine these hypotheses, we studied the effects of ion concentrations on the Na and Cl transport and I_{sc} in the preparations of rabbit ileum in HCO_3-free solutions. The choice of the HCO_3-free solutions was based on the previous observations by Binder *et al.* [3] that only in HCO_3-free solutions was there a significant Cl transport, and the I_{sc} represented approximately the algebraic sum of the net flux of Na and Cl.

As is well known, Na ions are continuously pumped into the intercellular spaces from the epithelial cell and then diffuse across the tight junction into the mucosal solution and across the lateral spaces into the serosal solution. It is apparent that the two barriers in the paracellular shunt pathway should have effects on the active Na movement across the intestinal mucosa and therefore the three-compartment model, first proposed by Koefoed-Johnsen and Ussing [16] for Na transport in frog skin, is probably inadequate for treatment of Na transport systems across epithelial tissues, especially in leaky epithelia such as rabbit ileum. A model containing parallel cellular and shunt compartments may be suitable for examining the effect of paracellular shunt pathway on the active Na movement across the rabbit ileum.

Materials and Methods

Male New Zealand white rabbits (ca. 2 kg) were killed by intravenous injection of 150 mg of sodium pentobarbital and the distal ileum was removed rapidly. The experimental techniques for transmural flux measurements were similar to those used in studies on guinea pig ileum *in vitro* [20]. In brief, the intestine was stripped of its serosal and muscle layers as previously described [20] and mounted as a flat sheet between two lucite half-chambers having an aperture of 1.13 cm^2. Identical solutions were circulated and oxygenated by appropriate gas on both sides of the tissue at 37 °C using the water-jacketed system described by Schultz and Zalusky [24]. The solutions were connected via agar bridges to calomel electrodes for measurement of electrical potential difference (PD) and to Ag – AgCl electrodes for passing current to the system in order to provide short-circuit conditions ($PD = 0$). The electrodes were connected to an automatic voltage clamp to maintain continuous short-

circuiting and the clamp was corrected for the fluid resistance between the *PD* sensing bridges.

Usually, 4 to 6 pieces of tissue from the same animal were studied simultaneously. Bidirectional Na fluxes were always determined by double labeled experiments using ^{22}Na and ^{24}Na. Unidirectional mucosa-to-serosa and serosa-to-mucosa Cl fluxes were measured on adjacent pieces of tissue using ^{36}Cl. After mounting, the tissue was rinsed twice with the appropriate solution, short-circuited, and radioactive isotopes were added to the mucosal and/or serosal solutions. Samples were taken at the beginning and the end of an experiment from the "hot" side, and after isotopes were added 4 or 6 samples were taken at a 20-min interval from the "cold" side. The tissues were kept short-circuited throughout the experiment, except for about 5 sec every 10 min for an open-circuit *PD* measurement.

The experimental procedures for determining intracellular Na, Cl and K concentrations were similar to those used by Schultz *et al.* [23]. In short, a segment of intestine was separated into a "mucosal strip" and a "serosal strip" using microscope glass slides as described by Dickens and Weil-Malherbe [9]. The mucosal strip, consisting of the epithelial layer and underlying connective tissue, was separated into small sheets which were incubated at 37 °C for a desired period of time in appropriate buffer solution containing ^{3}H-inulin as extracellular space marker or ^{22}Na for measuring exchangeable Na pool. The medium was bubbled with humidified O_2 or a mixture of 5% CO_2 and 95% O_2. The tissue was then taken out of the medium, gently blotted on Whatman No. 1 filter paper, and cut into two or three portions of approximately equal size, each of which was immediately weighed on a quartz-helix micro balance (Misco). One portion of the tissue was used for determination of the dry-to-wet weight ratio after drying at 90 °C for 24 hr. The other portions were extracted in 5 ml of 0.1 N HNO_3 with constant shaking for at least 2 hr. Aliquots of extract were assayed simultaneously for ^{22}Na and ^{3}H content using a liquid scintillation spectrometer (Nuclear Chicago Corp.), for Na and K content using a flame photometer, and for Cl content using a Buchler-Cotlove chloridometer.

The intracellular Na concentration obtained by using ^{22}Na is presumably measuring the "free" or "exchangeable" Na concentration in the epithelial cells and is calculated as

$$[^{22}Na]_c = [(cpm)_t^{Na} - ECS \cdot (cpm)_m^{Na}]/s.a./\text{cell } H_2O \text{ content} \quad (1)$$

$$ECS = (cpm)_t^{inulin}/(cpm)_m^{inulin} \quad (2)$$

$$s.a. = (cpm)_m^{Na}/[Na] \quad (3)$$

in which $(cpm)_t^{Na}$ and $(cpm)_t^{inulin}$ are the total counts per minute, cpm, of ^{22}Na and ^{3}H-inulin in the tissue, respectively, *ECS* is the extracellular spaces measured by ^{3}H-inulin, $(cpm)_m^{Na}$ and $(cpm)_m^{inulin}$ are the cpm of ^{22}Na and ^{3}H-inulin per unit volume in the bathing medium, respectively, *s.a.* is the specific activity of ^{22}Na in the medium, and the cell H_2O content is obtained by subtracting the dry weight and *ECS* from the wet weight of the tissue. The total intracellular Na concentration, $[Na]_c$, is obtained as the ratio of the difference between the total tissue Na content and the Na content in *ECS*, to the cell H_2O content. The "inexchangeable" intracellular Na concentration is defined as the difference between $[Na]_c$ and $[^{22}Na]_c$ or the residue intracellular Na concentration in Na-free solution.

The technique for separating the unidirectional transmural Na flux into a diffusional (presumably via a paracellular shunt pathway) and a nondiffusional (presumably via a transcellular pathway) component was similar to that used by Desjeux *et al.* [7], Frizzell and Schultz [14], and Schultz and Zalusky [23]. In brief, two adjacent pieces of tissue from the same animal were bathed in identical solution. ^{22}Na was added to the mucosal or serosal compartment and 20 min was allowed for reaching steady state under short-circuit conditions. Samples were taken at 10-min intervals from the "cold side" of one piece of the tissue in the order of $PD = 0, +5, +10, +10, +5, 0, -5, -10, -10, -5$, and 0, and the sequence of sampling in the other chamber was reversed. The unidirectional Na flux is plotted *vs.* $\exp(\pm F\Psi_{ms}/2RT)$,

in which Ψ_{ms} is potential difference across the tissue with the mucosal side taken as reference, F, R, and T have their usual meanings, $+F\Psi_{ms}/2RT$ is used for serosa-to-mucosa Na flux and $-F\Psi_{ms}/2RT$ is used for mucosa-to-serosa Na flux. The slope and intercept of the linear regression line represent the diffusional and the nondiffusional Na flux, respectively.

The standard HCO_3-free solution contains (mM): NaCl, 140; K_2HPO_4, 2.4; KH_2PO_4, 0.4; $CaCl_2$, 1.2; and $MgCl_2$, 1.2. The standard HCO_3-Cl-free solution was made by replacing NaCl with Na isethionate and $CaCl_2$ and $MgCl_2$ with the sulfate salts. The standard Na-free solution was prepared by replacing NaCl with choline Cl. HCO_3-free solutions with varying Na or Cl concentrations were prepared by mixing either one of the latter two solutions with the standard HCO_3-free solution in different proportions. The [Na], [Cl], and osmolarity of any solution were checked by flame photometer, chloridometer, and osmometer, respectively. All HCO_3-free solutions were bubbled with pure O_2 and gave a pH of 7.2–7.4.

Results

Effects of [Na] on Na and Cl Transport and I_{sc}

Experiments were carried out to measure simultaneously the bidirectional Na fluxes, I_{sc}, potential difference (PD), and tissue conductance (G) on the same pieces of tissue in solutions containing various [Na] at fixed [Cl]. The results are shown in Table 1. Fig. 1 presents the plot of the J^{Na}_{net} and I_{sc}, normalized with respect to the value of I_{sc} at 140 mM Na, *vs.* [Na]. Both J^{Na}_{net} and I_{sc} show similar saturation functions of Na and the difference between them is not significant for the [Na] from zero to 112 mM. This relationship suggests that the J^{Na}_{net} provides a measure of I_{sc}

Table 1. Unidirectional and net Na fluxes, I_{sc}, PD, and total tissue conductance (G) *vs.* [Na]

[Na]	J^{Na}_{ms}	J^{Na}_{sm}	J^{Na}_{net}	I_{sc}	PD	G
140 (11)	11.0±0.5	9.8±0.4	1.2±0.2[a]	3.0±0.2	3.8±0.4	22.4±1.2
112 (6)	11.0±0.4	8.3±0.4	2.8±0.3[b]	2.6±0.4	3.2±0.5	21.8±0.7
93 (6)	8.9±0.4	6.6±0.3	2.2±0.2	2.6±0.5	3.6±0.7	19.5±0.7
70 (5)	6.6±0.5	4.8±0.3	1.8±0.3	1.7±0.3	2.5±0.4	18.5±1.2
47 (6)	5.1±0.3	3.3±0.1	1.8±0.2	1.3±0.5	2.4±0.9	14.5±0.7
23 (5)	2.5±0.3	1.5±0.1	1.1±0.3	0.6±0.2	1.0±0.2	13.2±0.9
10 (5)	1.1±0.1	0.6	0.5±0.1	0.3±0.1	0.6±0.1	10.9±1.0

Values are means ±SE. [Na], Na fluxes and I_{sc}, PD, and G are in units of mM, μequiv/hr cm^2, mV, and mmho/cm^2, respectively. The number in parentheses gives number of animals. Four pieces of tissue from each of six animals were studied simultaneously in solutions containing 140, 112, 93, and 47 mM Na and from each of five animals were studied simultaneously in solutions containing 140, 70, 23, and 10 mM Na.

[a] Significantly different from the I_{sc} at 140 mM Na ($p<0.001$) by Student's t-test for paired variates.

[b] Significantly different from the J^{Na}_{net} at 140 mM Na ($p<0.025$) Student's t-test for paired variates.

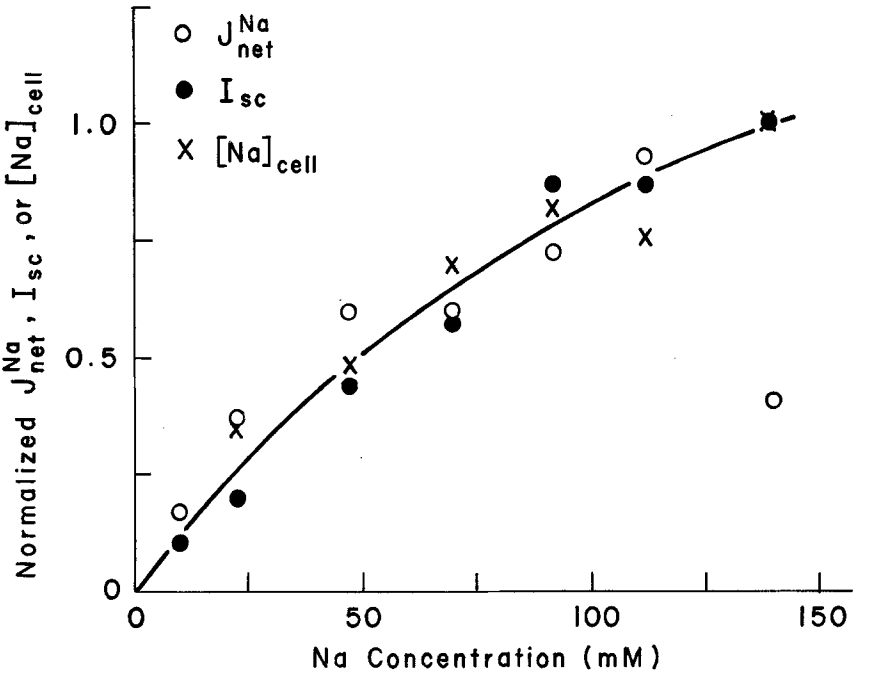

Fig. 1. Normalized J^{Na}_{net}, I_{sc}, and exchangeable intracellular [Na] *vs.* [Na]. J^{Na}_{net} and I_{sc} are normalized with respect to the mean value of I_{sc} at 140 mM Na, i.e., 3.0 μequiv/hr cm^2, and the exchangeable intracellular [Na] measured by ^{22}Na are normalized with respect to the value at 140 mM Na in the medium

and the Na ion may be the only ion species undergoing net transport across the rabbit ileum in this [Na] range. However, when [Na] is raised from 112 to 140 mM, there appears a sudden sharp drop in J^{Na}_{net} while I_{sc} increases smoothly. The difference between the J^{Na}_{net} and I_{sc} at this [Na] is highly significant ($p<0.001$).

Net Cl flux, J^{Cl}_{net}, was measured at three different [Na]. The J^{Cl}_{net} was found to be -0.08 ± 0.35 μequiv/hr cm^2 in three rabbits at 23 mM Na and 0.19 ± 0.27 μequiv/hr cm^2 in five rabbits at 70 mM Na. Neither value is significantly different from zero. A net secretion of Cl, -1.39 ± 0.50 μequiv/hr cm^2 ($p<0.05$), was observed in eight rabbits at 140 mM Na. Similar Cl secretion was observed previously under the same conditions [3]. It is important to note that the I_{sc} represents the algebraic sum of the net flux of Na and Cl for all [Na] and that the net secretion of Cl required high [Na]. It remains unclear from the experiments performed thus far whether or not the J^{Cl}_{net} contributes to the I_{sc}, and this will be clarified by the experiments listed below.

The nonlinear relationship between I_{sc} and [Na] is similar to that found in the guinea pig ileum [20] but differs from the linear relationship

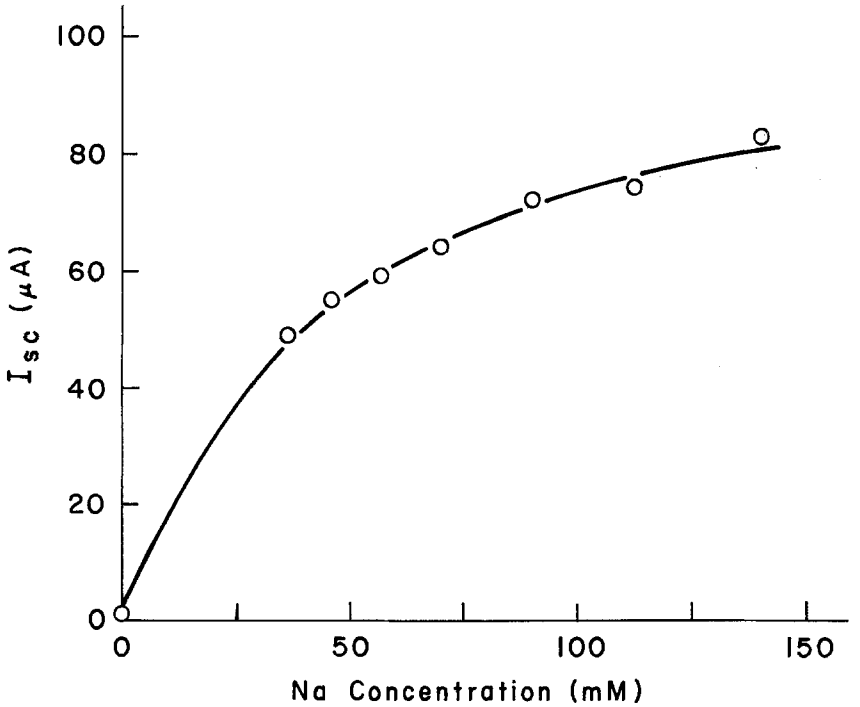

Fig. 2. Relationship between I_{sc} and [Na] obtained from measurements on a typical piece of tissue. The fluid resistance between the *PD*-sensing bridges was determined at various [Na] prior to mounting the tissue. [Na] was varied by mixing the standard HCO_3-free solution with the standard Na-free solution in different proportions. During the course of experiment, correction of fluid resistance was adjusted according to the [Na] present in the bathing solution

observed in rabbit ileum [24]. To further examine this relationship, measurements of I_{sc} *vs.* [Na] were carried out on a single piece of tissue. The results are shown in Fig. 2. The difference between the linear relationship between I_{sc} and [Na] found by Schultz and Zalusky [24] and the saturation function found by us may be attributed to variations in experimental conditions used. Their studies differed from ours in that the bathing solutions contained 2.5 mM HCO_3^- and at least 12 mM K; K rather than chlorine was used to replace Na; the fluid resistance between the *PD* sensing bridges was not corrected when measuring the I_{sc}; the system was not continuously short-circuited; and finally, the muscle layer was not stripped.

It seems likely that the high [K] in solutions with low [Na] is in part responsible for the linear relationship found. It has been shown that high concentrations of K will inhibit the ouabain-sensitive ($Na^+ - K^+$)-ATPase

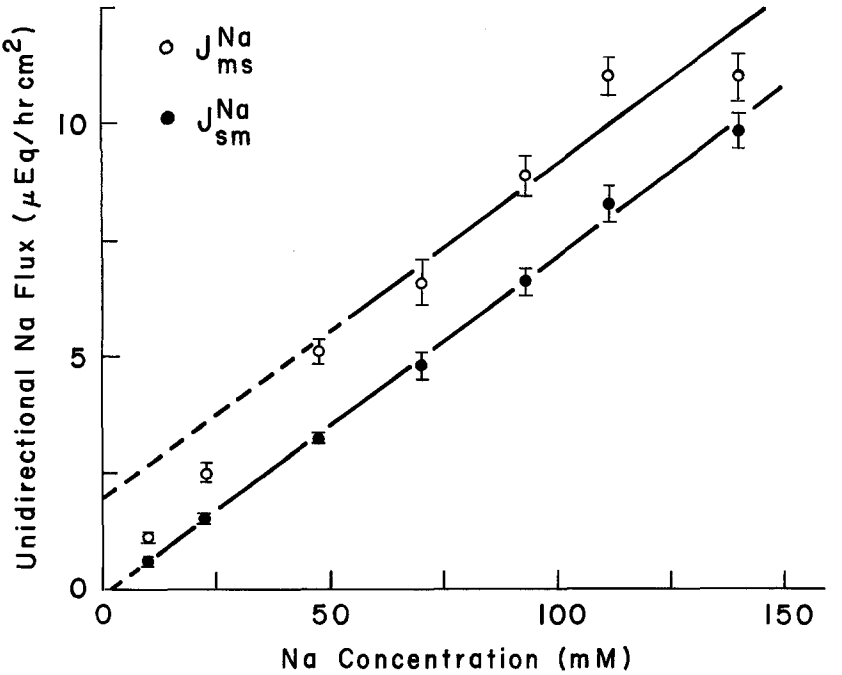

Fig. 3. Unidirectional mucosa-to-serosa and serosa-to-mucosa Na fluxes *vs.* [Na]. A least squares calculation gives $J^{Na}_{sm}=(0.072\pm0.002)[Na]+(-0.13\pm0.13)$ and the linear portion of $J^{Na}_{ms}=(0.071\pm0.012)[Na]+(1.99\pm1.21)$

activities in the plasma membranes isolated from the rat intestinal mucosa [23]. Thus the low I_{sc} in the low [Na] range perhaps resulted from the effect of high [K]. A similar saturable relationship between I_{sc} and [Na] has been observed in most other systems which have been investigated [6, 8, 13, 29], except a linear relationship is observed in rat colon [5].

The dependence of the unidirectional Na fluxes on [Na] is shown in Fig. 3. The mucosa-to-serosa Na flux, J^{Na}_{ms}, exhibits both a linear and a saturable component and the serosa-to-mucosa Na flux, J^{Na}_{sm}, appears to be a linear function of [Na]. The slope of the regression line of J^{Na}_{ms} over the [Na] range from 47 to 140 mM is 0.071 ± 0.012 cm/hr and of J^{Na}_{sm} is 0.072 ± 0.002 cm/hr. The slopes of the two lines in Fig. 3 do not differ significantly from each other indicating that the bidirectional diffusional Na fluxes are equal. This equality in diffusional Na fluxes is consistent with the concept that there should be no net diffusion of any ion across the tissue under short-circuit conditions in the steady state. It is also in agreement with the *PD* dependent diffusional Na fluxes via the paracellular shunt pathway described by Desjeux *et al.* [7]. The linear relationship between J^{Na}_{sm} and [Na] might suggest that the J^{Na}_{sm} is entirely attributable to simple ionic

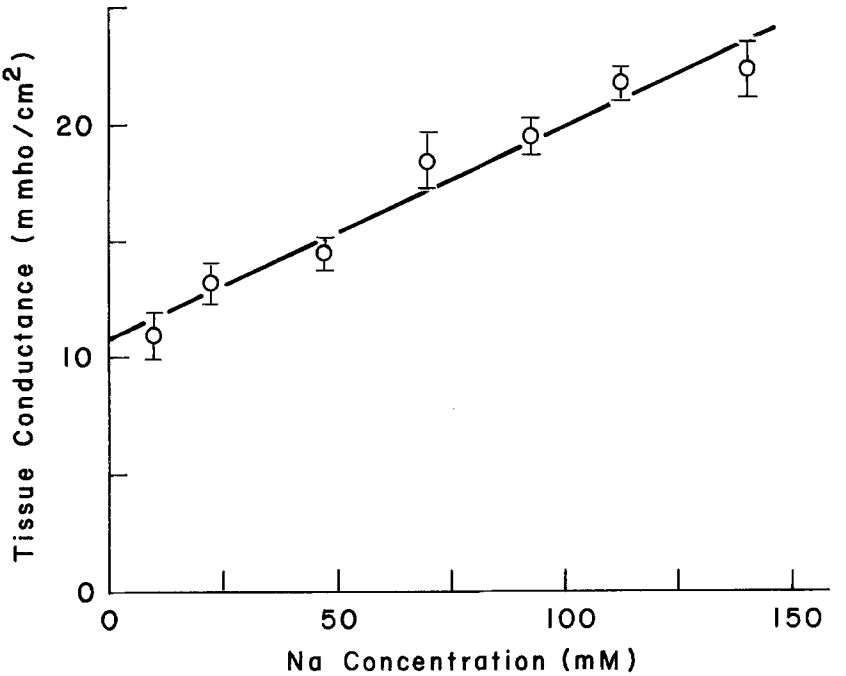

Fig. 4. Total tissue conductance *vs.* [Na]. A linear least squares calculation gives $G=(0.092 \pm 0.009)[\mathrm{Na}]+(10.8 \pm 0.7)$

diffusion [24]. However, the compelling evidence of a serosa-to-mucosa *PD* independent transcellular Na flux obtained by Desjeux *et al.* [7] indicates that the J_{sm}^{Na} at 140 mM Na contains two components. The question of whether the J_{sm}^{Na} at 140 mM is completely diffusional or contains two components may be clarified by the relationship between the tissue conductance and [Na], and by the relationship between the diffusional Na flux and [Na] determined in the next experiments. The relatively low tissue conductance at 140 mM Na, as shown in Table 1 and Fig. 4, provides some support to the idea that a declining transepithelial conductance at 140 mM Na results in a decreased passive Na flux in both directions across the rabbit ileum, but an increased transcellular Na flux offsets this such that J_{sm}^{Na} remains on the regression line.

Transcellular and Paracellular Na Movement

Since the relationship between the diffusional Na flux, J_{dms}^{Na}, and [Na] may explain whether or not the J_{sm}^{Na} is completely diffusional, and the relationship between I_{sc} and the transcellular Na flux from mucosa to

Table 2. Mucosa-to-serosa paracellular and transcellular Na fluxes

[Na]	${}_0J^{Na}_{ms}$	${}_0J^{Na}_{sm}$	J^{Na}_{dms}	J^{Na}_{cms}	I_{sc}
140 (5,10)	11.0±0.5	9.8±0.4	8.39±0.33	2.86±0.39	3.0±0.2
70 (5,10)	6.6±0.5	4.8±0.3	5.62±0.25	1.56±0.16	1.7±0.3

Values are means ±SE. [Na] and Na fluxes are in units of mM and μequiv/hr cm^2, respectively. Numbers in parentheses give number of animals followed by number of tissues. ${}_0J^{Na}_{ms}$, ${}_0J^{Na}_{sm}$, J^{Na}_{dms}, and J^{Na}_{cms} are the transmural mucosa-to-serosa and serosa-to-mucosa Na fluxes under short-circuit conditions, Na flux via the shunt, and Na flux via the transcellular pathway, respectively. Values of ${}_0J^{Na}_{ms}$, ${}_0J^{Na}_{sm}$, and I_{sc} are from Table 1.

serosa may provide information concerning the nature of I_{sc} and possible explanation for the difference between I_{sc} and J^{Na}_{net}, experiments were carried out to separate the unidirectional transmural Na fluxes from mucosa to serosa into a diffusional (presumably paracellular) and a nondiffusional (presumably transcellular) component in 10 pieces of tissue from 5 rabbits in solutions containing 70 and 140 mM Na. The results are summarized in Table 2 in terms of the mucosa-to-serosa transcellular and paracellular Na fluxes. The results of J^{Na}_{ms}, J^{Na}_{sm}, and I_{sc} under short-circuit conditions from Table 1 are also shown in Table 2 for comparison. The J^{Na}_{dms} at 140 mM Na is significantly less than twice the value of the J^{Na}_{dms} at 70 mM Na ($p<0.001$, using doubled values of both the mean and SE of the J^{Na}_{dms}), and is significantly less than the ${}_0J^{Na}_{sm}$ at 140 mM Na ($p<0.025$). The transcellular Na flux from mucosa to serosa, J^{Na}_{cms}, does not differ significantly from I_{sc} and the difference between the J^{Na}_{cms} and the J^{Na}_{net} at 140 mM Na is highly significant ($p<0.001$) and of similar value to the net Cl secretion, -1.39 μequiv/hr cm^2, and the transcellular serosa-to-mucosa Na flux, -1.15 μequiv/hr cm^2, obtained by Desjeux *et al.* [7]. The results strongly suggest that J^{Na}_{cms} represents I_{sc} and that the difference between the J^{Na}_{net} and I_{sc} at 140 mM is due to an onset of an "electrically neutral" NaCl flux from serosa to mucosa. In addition, the observation by Desjeux *et al.* [7] that the transcellular serosa-to-mucosa Na flux obtained under various conditions has no clear connection with I_{sc} also lends support to this suggestion.

Effects of [Cl] on Na and Cl Transport and I_{sc}

Further information regarding the nature of the difference between the J^{Na}_{net} and I_{sc} at 140 mM Na may be obtained from studies of the effects of [Cl] on Na and Cl transport and I_{sc}. Table 3 shows the dependence of the J^{Na}_{net} and I_{sc} on [Cl]. The results indicate that neither J^{Na}_{net} nor I_{sc} is strongly

Table 3. Na fluxes, I_{sc}, PD, and tissue conductance (G) *vs.* [Cl]

[Cl]	J^{Na}_{ms}	J^{Na}_{sm}	J^{Na}_{net}	I_{sc}	PD	G
145 (6)	9.1±0.3	8.0±0.3	1.1±0.4	2.0±0.2[a]	3.2±0.5	17.7±1.1
120 (6)	10.1±0.7	8.5±0.6	1.6±0.4	2.0±0.2	2.9±0.5	20.3±2.5
90 (6)	12.6±0.9	10.7±0.5	2.0±0.7	2.3±0.2	2.9±0.5	21.4±0.9
60 (6)	10.1±0.6	8.5±0.5	1.6±0.4	2.0±0.3	2.9±0.5	19.1±1.1
23 (6)	12.2±0.5	10.9±0.9	1.4±0.4	1.8±0.2	2.9±0.3	17.2±0.7
0 (6)	12.0±0.8	10.7±0.9	1.3±0.4	1.2±0.2	2.2±0.3	14.3±0.5

Values are means ±SE. *See* footnotes in Table 1 for units and the number in parentheses. [a] Significantly different from the I_{sc} at 145 mM Cl ($p<0.025$) by Student's t-test for paired variates. Six pieces of tissue from each of six animals were studied simultaneously in solutions containing various [Cl] at fixed [Na].

Table 4. Cl fluxes and I_{sc} *vs.* [Cl]

[Cl]	J^{Cl}_{ms}	J^{Cl}_{sm}	J^{Cl}_{net}	I_{sc}
145 (5)	9.53±0.62	10.82±0.80	−1.30±0.68	2.21±0.17
108 (5)	7.28±0.47	8.22±0.66	−0.73±0.51	2.27±0.19
72 (5)	5.48±0.38	5.77±0.47	−0.30±0.17	2.21±0.20
23 (5)	2.35±0.23	2.42±0.24	−0.07±0.20	1.75±0.15

Values are means ±SE. Cl fluxes and I_{sc}, and [Cl] are in units of μequiv/hr cm^2, respectively. Number in parentheses gives number of animals or tissue pairs. Negative sign for J^{Cl}_{net} designates secretion or net flux from serosa to mucosa.

affected by varying [Cl]. I_{sc} has virtually the same value for all solutions except the one completely free of Cl and is not significantly different from J^{Na}_{net} at all [Cl] but 145 mM Cl at which the difference is significant ($p<0.025$). Since two groups of experiments have been performed for measuring J^{Na}_{net} and I_{sc} in the standard HCO_3-free solution (presented in Tables 1 and 3), the pooled results give 1.2±0.2 and 2.7±0.2 μequiv/hr cm^2 for the J^{Na}_{net} and I_{sc}, respectively, and 1.5±0.2 μequiv/hr cm^2 for the difference between them ($p<0.001$). It appears that addition of Cl into a HCO_3-Cl-free solution can stimulate the Na transport systems.

The results of the J^{Cl}_{net} and I_{sc} determined at four different [Cl] are summarized in Table 4. The net secretion of Cl increases quite rapidly with increasing [Cl] while I_{sc} is not changed. The effects of [Cl] on Na and Cl transport and I_{sc} provide additional evidence for the suggestion that Cl transport is coupled to the movement of Na from serosa to mucosa and does not contribute to I_{sc}.

Effect of [K] on I_{sc}

The effect of [K] on the I_{sc} was evaluated on the tissues bathed in solutions contained approximately identical [Na] and [Cl] but different [K]. In each instance, I_{sc} was determined on 6 pieces of tissue from the same animal in solutions containing 0, 5.2, and 12.6 mM K. The time course of I_{sc} in these solutions is shown in Fig. 5. The I_{sc} did not differ significantly in the solutions containing 5.2 and 12.6 mM K but was significantly lower in the K-free solution especially in the second-hour period. The [K] in the "K-free solution" was 0.31 ± 0.02 mM at the end of the second hr. The effect of [K] on I_{sc} appears to be reversible as indicated by the slow recovery of the I_{sc} after addition of 5.2 mM K to the K-free solution at the end of the second hr. The effect of removal of K from the bathing solution in this study appears to be similar to the observations in the frog skin [29] and toad bladder [15] but different from the observation over a one-hour period in rabbit ileum with intact muscle layer [24].

The difference may be attributed to the very prolonged lag, especially in the preparations with intact muscle layer. No attempts were made to examine the effect of removal of K from the bathing solution on the Na and Cl transport because of this prolonged lag.

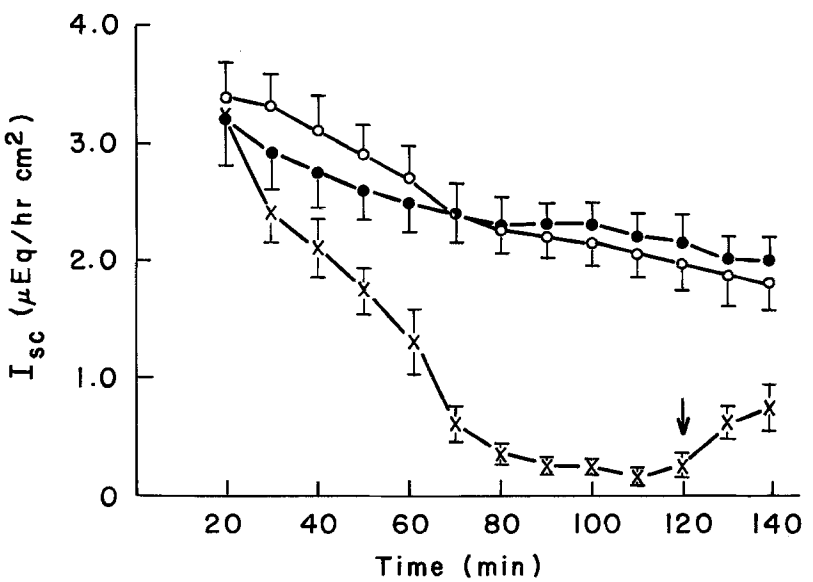

Fig. 5. Time course of I_{sc} in K-free solution (×) and solutions containing 5.2 mM K (●) and 12.6 mM K (○). At arrow, K^+ was added to the K-free solutions on both sides of the tissue to raise [K] to 5.2 mM

Intracellular Na, Cl, and K Concentrations

In the small intestine, as in almost all the other epithelia, active Na transport is thought to be closely related to the activities of the ouabain sensitive ($Na^+ - K^+$)-ATPase [20, 25]. Quigley and Gotterer [21] have shown that the relative activity of the ($Na^+ - K^+$)-ATPase in the plasma membranes isolated from rat small intestine is a saturable function of the [Na] in the medium. Since the Na ion is continuously pumped out of the epithelial cells to the serosal solution, it would be of interest to examine the intracellular Na, Cl, and K concentrations as functions of the ion composition in the external medium.

Table 5 summarizes the results of the intracellular Na, Cl, and K concentrations from the tissues exposed to the solutions containing 140 mM Na and various [Cl] and [HCO_3]. It appears that the intracellular Na and K concentrations do not vary significantly with the anion concentration in the medium. All the Cl-containing solutions give approximately the same intracellular Cl concentration and the Cl-free solutions give about 10 mM inexchangeable or slowly exchangeable Cl. A constant intracellular Na concentration was also observed by Koopman and Schultz [17] in preparations of rabbit ileum exposed to solutions containing various sugars and amino acids.

The results of the intracellular Na, Cl, and K concentrations as functions of the [Na] in the HCO_3-free solutions are shown in Table 6. Again, the intracellular Cl and K concentrations do not change with the external [Na] but the intracellular Na concentration shows a saturation function of the external [Na]. ^{22}Na was used to determine the exchangeable

Table 5. Intracellular [Na], [Cl], and [K] in various bathing solutions containing 140 mM Na

Solution	Cell water/dry weight	$[Na]_c$	$[Cl]_c$	$[K]_c$
IS (3,9)	3.51±0.19	79±4	84±3	135± 7
Ringer's (6,12)	3.48±0.17	82±6	70±4	122± 8
HCO_3-free (5,13)	3.38±0.13	78±5	73±2	124± 7
Cl-free (3,7)	3.33±0.25	79±8	10±1	107±15
HCO_3 − Cl-free (5,13)	3.19±0.12	79±3	11±1	119± 6

Values are means ±SE. Cell water is the tissue corrected for the inulin space. Intracellular ion concentrations are in units of mmole per 1 kg cell water. IS solution contains 140 mM NaCl, 10 mM $KHCO_3$, 1.2 mM K_2HPO_4, 0.2 mM KH_2PO_4, and 1.2 mM $CaCl_2$ and $MgCl_2$. Ringer's solution contains 115 mM NaCl, 25 mM $NaHCO_3$, 2.4 mM K_2HPO_4, 0.4 mM KH_2PO_4, and 1.2 mM $CaCl_2$ and $MgCl_2$. Cl-free solution has the same salt composition as the Ringer's solution except all Cl^- is replaced by isethionate. HCO_3-free and HCO_3 − Cl-free solutions are the standard HCO_3-free and HCO_3 − Cl-free solutions described in Methods.

Table 6. Intracellular [Na], [Cl], and [K] *vs.* [Na] in HCO_3-free bathing solution

[Na]	$[Na]_c$	$[^{22}Na]_c$	$[Cl]_c$	$[K]_c$
140 (3,6)	73±9	65±9	73±4	117±11
112 (3,6)	62±9	49±9	73±4	116±13
93 (3,6)	71±7	53±5	76±3	104±10
70 (3,6)	57±6	45±5	77±5	106±11
47 (3,6)	43±4	31±3	75±4	113± 7
23 (3,6)	38±5	22±2	82±3	100±10
2 (3,6)	19±3	4	83±9	105± 6

Values are means ±SE. Numbers in parentheses give number of animals followed by number of tissues. $[^{22}Na]$ is the intracellular [Na] measured by ^{22}Na.

intracellular Na concentration. The results indicate that approximately 10 to 20 mM Na is inexchangeable. The exchangeable intracellular Na concentration normalized with respect to the value at 140 mM external [Na] is shown in Fig. 1 along with the J_{net}^{Na} and I_{sc}. It appears that the intracellular Na concentration bears the same relationship as the J_{net}^{Na} and I_{sc} with the external [Na]. This relationship suggests that there is a large, measurable Na pool which is related to transepithelial Na transport.

Discussion

Active Na and Cl Transport

The similarity between the J_{net}^{Na} and I_{sc} and the failure to observe a significant net transport of Cl in the [Na] range of 0 to 112 mM indicate that Na^+ is the only ion species undergoing net transport under short-circuit conditions. The finding (Table 2) that the transcellular mucosa-to-serosa Na flux and I_{sc} do not differ significantly provides compelling evidence that the active absorption of Na is electrogenic and responsible for the I_{sc}. It seems unlikely that the sharp drop in J_{net}^{Na} as [Na] is increased from 112 to 140 mM is caused by an inhibition of the active Na absorption. An onset of coupled NaCl secretion at this [Na] could account for both the sharp decrease in J_{net}^{Na} and the significant difference between the I_{sc} and J_{net}^{Na}. The proposed serosa-to-mucosa coupled NaCl flux is supported by the similarity in the magnitude of a *PD* independent serosa-to-mucosa transcellular Na flux of -1.15 μequiv/hr cm^2 observed by Desjeux *et al.* [7] and the magnitude of net Cl secretion of -1.39 μequiv/hr cm^2 observed in this study and by Binder *et al.* [3]. The results in Table 4 indicating that [Cl] changes the J_{net}^{Cl} but has virtually no effect on I_{sc} and the findings by

Desjeux *et al.* [7] that the transcellular serosa-to-mucosa Na flux obtained under various conditions has no necessary connection with the I_{sc} provide additional evidence to the suggestions that the serosa-to-mucosa Na and Cl fluxes are coupled and do not contribute to I_{sc}.

Based on these considerations, the patterns of Na and Cl transport in HCO_3-free solution may be summarized as follows: (a) the active absorptive Na process is electrogenic and accounts for I_{sc} and (b) the secretory NaCl flux requires high [Na], varies with [Cl], and does not contribute to I_{sc}. Basically, these conclusions are in agreement with the two transport systems model proposed by Powell *et al.* [20] to explain the patterns of electrolyte transport in guinea pig ileum.

Although these results suggest a specific NaCl secretory process, they provide no information on the location or routes of this flux. If the flux occurs mainly through the villous cells that are thought to be responsible for ion and water absorption, it may be closely associated with the rheophylline-sensitive, coupled NaCl influx at the brush border proposed by Nellans, Frizzell and Schultz [19]. The authors suggested that the coupled NaCl influx was reversible and hence capable of mediating NaCl efflux from cell to mucosal solution.

Effect of the Paracellular Shunt Pathway on Active Na Absorption

Schultz and Zalusky [23] and Clarkson [4] have developed the concept of two parallel pathways for Na movement across the intestinal mucosa, one active transport and one passive. The studies of Rose and Schultz [22] and Frizzell and Schultz [14] 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. As is well known, the right junction and lateral space between cells form two barriers in a series for solutes to diffuse across the low resistance shunt pathway. The relationship between net Na transport, I_{sc}, and intracellular Na concentration (Fig. 1) and the lack of I_{sc} (and presumably net Na transport) after prolonged exposure to K-free solution indicate that Na absorption by the ileal mucosa does occur from a measurable Na pool via a $(Na^+ - K^+)$-stimulated ATPase. Stirling [27] has shown in a radioautographic study of ^3H-ouabain binding to rabbit small intestine that the Na pump is located at the serosal or lateral membrane. This localization of

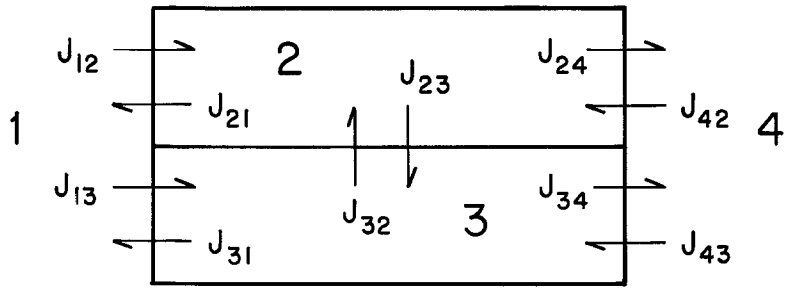

Fig. 6. A four-compartment model to approximate the Na transport systems in rabbit ileum. Compartments 1, 2, 3, and 4 refer to the mucosal solution, cell, paracellular shunt pathway, and the serosal solution, respectively. J_{ij} is the Na flux from compartment i to compartment j. J_{23} and J_{24} are active Na fluxes which result from the Na pump located at the serosal or lateral membrane

the Na pump fits the view that Na is continuously pumped into the intercellular spaces and then diffuses across the tight junction into mucosal solution and across the lateral spaces into serosal solution. It is, therefore, apparent that the two barriers in the shunt pathway affect active Na movement across the intestinal mucosa. A four-compartment model instead of the three-compartment model, first proposed by Koefoed-Johnson and Ussing [16] for Na transport in frog skin, is used to treat the Na transport systems across the intestine and to examine the effects of the shunt on active Na absorption. As shown in Fig. 6, compartments 1, 2, 3, and 4 refer to the mucosal solution, cell, shunt, and serosal solution, respectively, J_{ij} is the Na flux from compartment i to compartment j, and J_{23} and J_{24} are the active Na fluxes resulted from the Na pump. When identical solutions are present in compartments 1 and 4, and assuming the permeability of passive Na movement across any membrane is symmetrical, i.e., $P_{ij}=P_{ji}$, the net Na flux, J_{net}, across the tissue in the steady state can be expressed as (*see* Appendix)

$$J_{net}=[(J_{12}J_{24}-J_{42}J_{21})(J_{31}+J_{32}+J_{34})+J_{23}J_{32}J_{24} +J_{12}J_{23}J_{34}-J_{23}J_{32}J_{21}-J_{42}J_{23}J_{31}]/\text{Det}. \quad (4)$$

Since no information about the Na permeability, P_{32} or P_{42}, is available, and it has recently been verified that the inward-facing barrier of frog skin has a very low passive permeability to Na [2], we therefore make a further assumption that the lateral membrane is not permeable to passive Na movement, i.e., $P_{32}=P_{42}=0$. The net Na flux then represents Na absorption and becomes

$$J_{net}=J_{24}+J_{23}P_{34}/(P_{34}+P_{31}). \quad (5)$$

This expression illustrates that net Na absorption is reduced from the active Na pumping rate, $J_{23}+J_{24}$, by the amount of $J_{23}\,P_{31}/(P_{34}+P_{31})$. The Na absorptive flux can be equal to the pumping rate only when $P_{34} \gg P_{31}$. Desjeux *et al.* [7] have estimated that the tight junction contributes a minimum of 85–90% of the resistance of the shunt pathway to Na. Calculation of $P_{31}/(P_{34}+P_{31})$ using the values estimated by Desjeux *et al.* [7], approximately 4–11% of the Na flux from the cellular compartment to the lateral spaces diffuses back to the mucosal solution across the tight junction.

An interesting implication of Eq. (5) can be noted below. Since one of the conclusions indicates that I_{sc} is represented by the Na absorptive rate, changes in the ratio of $P_{31}/(P_{34}+P_{31})$ will cause changes in I_{sc} assuming J_{23} is unchanged. As we have often observed during experiments, I_{sc} slowly decreases with time in the "steady state" and these changes are usually accompanied by slow increases in total tissue conductance. Since the conductance of the shunt pathway contributes 85–90% of the total tissue conductance, changes in the resistance of the components of shunt pathway will alter the total tissue resistance. The permeability of the shunt pathway to Na, P_s, can be described by

$$\frac{1}{P_s}=\frac{1}{P_{TJ}}+\frac{1}{P_{LS}} \tag{6}$$

where P_{TJ} and P_{LS} are the permeability coefficients of the tight junction and lateral space to Na and equivalent to P_{31} and P_{34}, respectively. Estimates of P_{LS} can be made as outlined by Smulders, Tormey, and Wright [26] from the relation

$$P_{LS}=DA/l \tag{7}$$

where D is the diffusion coefficient of Na, A is the area of the lateral spaces per unit serosal area, and l is length of the spaces. It is apparent that increases in the dimensions of the lateral spaces will increase the total tissue conductance whereas decreases in the dimensions of the lateral spaces will result in decreases in the total tissue conductance. It is also apparent that in increase in P_{TJ} with P_{LS} unchanged should, by increasing the ratio of $P_{31}/(P_{34}+P_{31})$, cause a decrease in I_{sc} and an increase in tissue conductance whereas an increase in P_{LS} with P_{TJ} unchanged would result in an increase in I_{sc} and tissue conductance. These analyses suggest that the experimentally observed decreases in I_{sc} along with increases in tissue conductance may have resulted from a slowly developing leak in the tight junction to passive Na movement during the course of experiment.

In the steady state, the Na concentration in the cell and in the intercellular space can be expressed (*see* Appendix) as

$$C_2 = C_1 - (J_{23} + J_{24})/P_{12} < C_1 \tag{8}$$

$$C_3 = C_1 + J_{23}/(P_{31} + P_{34}) > C_1 \tag{9}$$

as $P_{32} = P_{42} = 0$. The results are that $C_2 < C_1$ and $C_3 > C_1$ are just what we have expected. However, quantitative evaluation of the Na concentrations in the cell and in the intercellular space is not possible without information regarding those permeability coefficients.

Appendix

We wish to examine the effect of low resistance shunt pathway on active Na absorption according to the model shown in Fig. 6 under short-circuited conditions in steady state. When radioactive tracers are initially present in compartment 1 (mucosal solution), the steady state rate of changes of tracers in other compartments can be expressed as

$$\frac{dP_2}{dt} = J_{12}\, p_1^* + J_{32}\, p_3^* - (J_{21} + J_{23} + J_{24})\, p_2^* = 0 \tag{A1}$$

$$\frac{dP_3}{dt} = J_{13}\, p_1^* + J_{23}\, p_2^* - (J_{31} + J_{32} + J_{34})\, p_3^* = 0 \tag{A2}$$

$$\frac{dP_4}{dt} = J_{24}\, p_2^* + J_{34}\, p_3^* \tag{A3}$$

where P_i and p_i^* are, respectively, the total amount of tracer and the specific activity in compartment i. P_i, p_i^*, and J_{ij} are expressed in units of counts, counts per unit time per unit amount of Na, and amount of Na per unit time, respectively. p_2^* and p_3^* can be expressed in terms of p_1^* by solving Eqs. (A1) and (A2)

$$p_2^* = p_1^* [J_{12}(J_{31} + J_{32} + J_{34}) + J_{23}\, J_{32}]/\mathrm{Det} \tag{A4}$$

$$p_3^* = p_1^* [J_{13}(J_{21} + J_{23} + J_{24}) + J_{12}\, J_{23}]/\mathrm{Det} \tag{A5}$$

where Det-$(J_{21} + J_{23} + J_{24})(J_{31} + J_{32} + J_{34}) - J_{23}\, J_{32}$. The unidirectional transmural Na flux can then be obtained as

$$\begin{aligned} J_{14} &\equiv \frac{1}{p_1^*}\frac{dP_4}{dt} \\ &= \{J_{24}[_{12}(J_{31} + J_{32} + J_{34}) + J_{23}\, J_{32}] + J_{34}[J_{13}(J_{21} + J_{23} + J_{24}) \\ &\quad + J_{12}\, J_{23}]\}. \end{aligned} \tag{A6}$$

Similarly, when tracers are initially present in compartment 4 (serosal solution), the transmural Na flux from serosa to mucosa is

$$J_{41} = \{J_{21}[J_{42}(J_{31}+J_{32}+J_{34})+J_{23}J_{32}]+J_{31}[J_{43}(J_{21}+J_{23}+J_{24})+J_{42}J_{23}]\}/\text{Det.} \tag{A7}$$

The net Na is

$$\begin{aligned} J_{\text{net}} &= J_{14}-J_{41} \\ &= \{(J_{12}J_{24}-J_{42}J_{21})(J_{31}+J_{32}+J_{34})+(J_{13}J_{34}-J_{43}J_{31})(J_{21} \\ &\quad +J_{23}+J_{24})+J_{23}J_{32}J_{24}+J_{12}J_{23}J_{34}-J_{23}J_{32}J_{21} \\ &\quad -J_{42}J_{23}J_{31}\}/\text{Det.} \end{aligned} \tag{A8}$$

The expression for J_{net} can be simplified by assuming any barrier or membrane is symmetrical to passive Na movement, i.e., $P_{ij}=P_{ji}$ and using $P_{ij}=J_{ij}/C_i$ in which P_{ij} is the permeability coefficient of Na from compartment i to compartment j and C_i is the Na concentration in compartment i. The second term in the numerator of Eq. (A8) can be nullified as following:

$$J_{13}J_{34}-J_{43}J_{31}=C_1\,C_3(P_{13}P_{34}-P_{43}P_{31})=0$$

as $C_1=C_4$. J_{net} then becomes

$$\begin{aligned} J_{\text{net}} = \{&(J_{12}J_{24}-J_{42}J_{21})(J_{31}+J_{32}+J_{34})+J_{23}J_{32}J_{24} \\ &J_{12}J_{23}J_{34}-J_{23}J_{32}J_{21}-J_{42}J_{23}J_{31}\}/\text{Det.} \end{aligned} \tag{A9}$$

In the steady state, the conservation of mass holds in compartments 2 and 3 so that

$$J_{21}+J_{23}+J_{24}=J_{12}+J_{32}+J_{42} \tag{A10}$$

$$J_{31}+J_{32}+J_{34}+J_{13}+J_{23}+J_{43}. \tag{A11}$$

The steady state Na concentration in compartments 2 and 3 can be derived from Eqs. (A10) and (A11) as

$$C_3=[(P_{31}+P_{34})\,C_1+J_{23}]/(P_{31}+P_{32}+P_{34}) \tag{A12}$$

$$C_2=C_1(P_{12}+P_{42})/P_{12}+C_3P_{32}/P_{12}-(J_{23}+J_{24})/P_{12}. \tag{A13}$$

Assuming $P_{32}=P_{42}=0$, we get

$$C_3=C_1+J_{23}/(P_{31}+P_{34})>C_1 \tag{A14}$$

$$C_2=C_1-(J_{23}+J_{24})/P_{12}<C_1. \tag{A15}$$

This work was supported by the United States-Japan Cooperative Medical Science Program administered by the National Institute of Allergy and Infectious Diseases (Grant AI-09277) and by United States Public Health Service Grants (AM-12028 and AM-17433) from the National Institute of Arthritis, Metabolism, and Digestive Diseases. Dr. Desjeux was supported in part by a fellowship from the French Foreign Ministry. Dr. Danisi was supported in part by a grant from Fundacao de Amparo a Pesquisa do Estado de Sao Paulo (Processo Biologicas 72/574).

References

1. Barry, R. J. C., Smyth, D. H., Wright, E. M. 1965. Short-circuit current and solute transfer by rat jejunum. *J. Physiol.(London)* **181**:410
2. Biber, T. U. L., Aceves, J., Mandel, L. J. 1972. Potassium uptake across serosal surface of isolated frog skin epithelium. *Am. J. Physiol.* **22**:1366
3. Binder, H. J., Powell, D. W., Tai, Y.-H., Curran, P. F. 1973. Electrolyte transport in rabbit ileum. *Am. J. Physiol.* **225**:776
4. Clarkson, T. W. 1967. The transport of salt and water across isolated rat ileum. *J. Gen. Physiol.* **50**:695
5. Curran, P.F., Schwartz, G.F. 1960. Na, Cl, and water transport by rat colon. *J. Gen. Physiol.* **43**:555
6. Curran, P.F., Solomon, A.K. 1957. Ion water fluxes in the ileum of rats. *J. Gen. Physiol.* **41**:143
7. Desjeux, J.-F., Tay, Y.-H., Curran, P. F. 1974. Characteristics of sodium flux from serosa to mucosa. *J. Gen. Physiol.* **64**:274
8. Diamond, J. M. 1962. The mechanism of solute transport by the gallbladder. *J. Physiol. (London)* **161**:474
9. Dickens, F., Weil-Malherbe, H. 1941. The metabolism of intestinal mucous membrane. *Biochem. J.* **35**:7
10. Field, M. 1971. Ion transport in rabbit ileal mucosa. II. Effects of cyclic-3′-5′-AMP. *Am. J. Physiol.* **221**:992
11. Field, M., Fromm, D., Al-Awqati, Q., Greenough III., W. B. 1972. Effect of cholera enterotoxin on ion transport across isolated ileal mucosa. *J. Clin. Invest.* **51**:796
12. Field, M., Fromm, D., McColl, I. 1971. Ion transport in rabbit ileal mucosa. I. Na and Cl fluxes and short-circuit current. *Am. J. Physiol.* **220**:1388
13. Frazier, H. S., Dempsey, E. F., Leaf, A. 1962. Movement of sodium across the mucosal surface of the isolated toad bladder and its modification by vasopressin. *J. Gen. Physiol.* **45**:529
14. Frizzell, R. A., Schultz, S. G. 1972. Ionic conductances of extracellular shunt pathway in rabbit ileum. *J. Gen. Physiol.* **59**:318
15. Hays, R.M., Leaf, A. 1961. The problem of clinical vasopressin resistance: *In vitro* studies. *Ann. Int. Med.* **54**:700
16. Koefoed-Johnson, V., Ussing, H. H. 1958. The nature of the frog skin potential. *Acta Physiol. Scand.* **25**:150
17. Koopman, W., Schultz, S. G. 1969. The effect of sugars and amino acids on mucosal sodium ion and potassium ion concentrations in rabbit ileum. *Biochim. Biophys. Acta* **173**:338
18. Leaf, A., Anderson, J., Page, L. B. 1958. Active sodium transport by the isolated toad bladder. *J. Gen. Physiol.* **41**:657
19. Nellans, H. N., Frizzell, R. A., Schultz, S. G. 1974. Brush border processes and trans-epithelial Na and Cl transport by rabbit ileum. *Am. J. Physiol.* **226**:1131

20. Powell, D. W., Binder, H. J., Curran, P. F. 1972. Electrolyte secretion by the guinea pig ileum *in vitro*. *Am. J. Physiol.* **223**:531
21. Quigley, J. P., Gotterer, G. S. 1969. Properties of a high specific activity, $(Na^+ - K^+)$-stimulated ATPase from rat intestinal mucosa. *Biochim. Biophys. Acta* **173**:469
22. Rose, R. C., Schultz, S. G. 1971. Studies on the electrical potential profile across rabbit ileum. *J. Gen. Physiol.* **57**:639
23. Schultz, S. G., Curran, P. F., Chez, R. A., Fuisz, R. E. 1967. Alanine and sodium fluxes across the mucosal border of rabbit ileum. *J. Gen. Physiol.* **50**:1241
24. Schultz, S. G., Zalusky, R. 1964. Ion transport in isolated rabbit ileum. I. Short-circuit current and Na fluxes. *J. Gen. Physiol.* **47**:567
25. Schultz, S. G., Zalusky, R. 1964. Ion transport in isolated rabbit ileum. II. The interaction between active sodium and active sugar transport. *J. Gen. Physiol.* **47**:1024
26. Smulders, A. P., Tormey, J. McD., Wright, E. M. 1972. The effect of osmotically induced water flows on the permeability and ultrastructure of the rabbit gall bladder. *J. Membrane Biol.* **7**:164
27. Stirling, C.E. 1972. Radioautographic localization of sodium pump sites in rabbit intestine. *J. Cell Biol.* **53**:704
28. Taylop, A.E., Wright, E.M., Schultz, S.G., Curran, P.F. 1968. Effects of sugars on ion fluxes in intestine. *Am. J. Physiol.* **214**:836
29. Ussing, H.H. 1960. The alkali metal ions in isolated systems and tissues. *In:* The Alkali Metal Ions in Biology, Handbuch der Experimentellen Pharmakologie. O. Eichler and A. Farah, editors. pp. 1–195. Springer-Verlag, Berlin
30. Ussing, H.H., Zerahn, K. 1951. Active transport of sodium as the source of electric current in the short-circuited frog skin. *Acta Physiol. Scand.* **23**:110