Ion Transport in Isolated Rabbit Ileum

I. *Short-circuit current and Na fluxes*

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ABSTRACT The transmural potential difference, short-circuit current, and Na fluxes have been investigated in an *in vitro* preparation of isolated rabbit ileum. When the tissue is perfused with a physiological buffer, the serosal surface is electrically positive with respect to the mucosal surface and the initial potential difference in the presence of glucose averages 9 mv. Unidirectional and net Na fluxes have been determined under a variety of conditions, and in each instance, most if not all of the simultaneously measured short-circuit current could be attributed to the active transport of Na from mucosa to serosa. Active Na transport is dependent upon the presence of intact aerobic metabolic pathways and is inhibited by low concentrations of ouabain in the serosal medium. A method is described for determining whether a unidirectional ionic flux is the result of passive diffusion alone, in the presence of active transport of that ion in the opposite direction. Using this method we have demonstrated that the serosa-to-mucosa flux of Na may be attributed to passive diffusion with no evidence for the presence of carrier-mediated exchange diffusion or the influence of solvent-drag.

The ability of the small intestine to absorb NaCl and water has been extensively documented using both *in vivo* and *in vitro* preparations (1, 2). The primary transport processes involved, however, are as yet the subjects of controversy. Curran and Solomon have presented evidence that both Na and Cl are actively transported by the *in vivo* rat ileum, and that water absorption is passive and dependent upon net solute flux (3). The experiments of Clarkson *et al.* (4–6), using an *in vitro* preparation of rat ileum, indicate that both Cl and water are passively transported and dependent upon the active transport of Na. However, since unidirectional Cl fluxes were not measured, the passive nature of Cl transport was not definitively established. Vaughn and Alpern (7) and Smyth and Taylor (8), on the other hand, have suggested that these observations may be explained equally well by the presence of active water transport with NaCl absorption following passively.

The study of ion transport across epithelial membranes has been greatly

facilitated by the short-circuit technique originally employed in the study of Na transport by the frog skin. Since its introduction by Ussing and Zerahn (9) this technique has been applied to the investigation of ion transport in toad bladder (10), toad (11) and frog (12) colon, and guinea pig cecum (11). The present paper is concerned with the relationships among Na fluxes, the transmural potential difference, and the short-circuit current in *in vitro* segments of isolated rabbit ileum.

METHODS

Experimental Procedure

New Zealand white rabbits, weighing approximately 2.5 to 4 kg, were anesthetized by the intravenous administration of nembutal. All animals had been quarantined for at least 1 month and were maintained on normal food intake up to the time of sacrifice. After opening the abdomen, the terminal 5 to 8 cm of ileum, clearly defined distally by the ileocecal junction and proximally by a characteristic patch of lymphoid tissue, was excised. The excised segment was then immersed in a beaker containing the experimental medium and rapidly opened by cutting along the mesenteric border. The exposed mucosal surface was rinsed free of intestinal contents and the tissue clamped between two lucite half-chambers. In most cases the animal was alive at the time of excision, and care was exercised to maintain an uncompromised blood supply to the intestinal segment. In some instances, the rabbit succumbed following the administration of nembutal, and the ileal segment was excised within 2 minutes after death. The elapsed time between excision and the onset of perfusion rarely exceeded 4 minutes. All manipulations up to the time of perfusion were performed at room temperature.

Perfusion of each surface of the tissue was accomplished employing a gas-lift circulating system which provided adequate aeration with a water-saturated 95 per cent O_2, 5 per cent CO_2 gas mixture. The perfusing solutions were maintained at 38.5°C by means of water jackets which were connected to a constant temperature circulating pump. The medium reservoirs were capped with glass condensors in order to minimize evaporative losses. In this way the decrease in the volume of the perfusate was limited to 3 to 5 per cent over a 60 minute period.

The composition of the bathing solution was NaCl 137 mM, KCl 5.0 mM, $CaCl_2$ 2.5 mM, $MgCl_2$ 2.2 mM, Na_2HPO_4 1.1 mM, KH_2PO_4 0.2 mM, and $NaHCO_3$ 2.5 mM. In some experiments glucose was present at a concentration of 11 mM. The pH was initially 7.2 and declined to 6.8 over a period of 45 to 60 minutes.

Electrical Measurements

The tips of Ringer-agar bridges (2.6 mm ID) were placed within 1 mm of each surface of the membrane at the center, and the potential difference (PD) between these bridges was measured by a pair of matched calomel electrodes leading into a high-impedance vacuum tube potentiometer (Kiethley, model 600A). The calomel electrode and bridge junction potentials measured in the experimental medium prior to mounting the membrane did not exceed 0.2 mv.

The external current was applied to the system employing immersed Ag-AgCl electrodes connected to a variable electromotive force. The magnitude of the applied current was determined by means of a Weston DC (model 622) microammeter. The area of exposed tissue in the lucite half-cells was 1.13 cm^2 and the distance from the center of this area to the Ag-AgCl electrode was 22 mm. Thus the difference in distances from the electrode to different points on the tissue did not exceed 1 mm, and with a current density of 150 $\mu a/cm^2$ the PD across different areas of the tissue would not differ by more than 0.8 mv.

The determination of the short-circuit current, I_{sc} (the external current required to abolish the transmural PD when both sides of the tissue are bathed with identical solutions) in the rabbit ileum is complicated by the low tissue resistance, so that the resistance of the perfusion solution between the tissue and the bridge tips contributes significantly to the total resistance of the system. To obtain the true transmural PD when an external current is applied to the system, correction for the potential drop between the bridge tips and the tissue surface must be made. In order to evaluate the magnitude of this correction, the DC resistance of the fluid gap between the bridge tips was measured in the absence of the tissue and with rapid circulation of the solution. These measurements were made over the range of PD's encountered in our studies, and at each increment the current rapidly assumed a stable level which was linearly related to the PD. The specific resistance of the perfusion medium at 38.5°C is 30 ohm-cm, thus the resistance of the 3 mm gap between the bridge tips and the tissue surface is 9 ohm-cm^2. Since the total DC resistance of fluid plus tissue averages 70 ohm-cm^2, the correction for fluid resistance is approximately 13 per cent, and, because the tissue behaves as a linear resistor, this correction factor is a constant. When the system is short-circuited (*i.e.* when the potentiometer needle is at zero), a small transmural PD persists, and the measured I_{sc} is 13 per cent lower than the true I_{sc}. It will be shown that our conclusions are not significantly altered by this constant error, and, for this reason, I_{sc} in the remainder of this paper will refer to the uncorrected current measured when the potentiometer reading is zero. It should be noted, however, that with low resistance tissues, such a rabbit ileum, results obtained using solutions having high specific resistances must be interpreted with caution. For example, if mannitol replaces NaCl in the present buffer the resulting solution has a specific resistance of 177 ohm-cm at 38.5°C and the resistance of the fluid layer is 53 ohm-cm^2; a value which is 80 per cent of the tissue resistance.

Na Fluxes

Unidirectional Na fluxes, from mucosa to serosa and serosa to mucosa, were determined individually using Na^{24} in single label experiments, and simultaneously by double labeling with Na^{24} and Na^{22}. In most experiments in which only Na^{24} was used the two unidirectional fluxes were determined at the same time on adjacent portions of tissue from the same animal by employing two identical chambers, etc. connected in parallel to the same constant temperature circulating pump. The short-circuit currents and PD's measured on each of these tissues differed by an average of 8 per cent, and in 75 per cent of the experiments they differed by less than 5 per cent. This difference may be attributed to the difficulty in controlling the degree of distension of the tissue during mounting in the chamber.

In the single label experiments, for example the determination of the mucosa to serosa flux, a tracer quantity of Na^{24} was added to a known volume of medium in the mucosal reservoir. After a period of 15 to 20 minutes, which is required to achieve a steady-state flux, 1.0 ml aliquots were withdrawn from the serosal reservoir at 5 to 10 minute intervals. After each withdrawal, 1.0 ml of fresh medium was added to the reservoir to maintain a constant volume. Aliquots were withdrawn from the mucosal reservoir at the beginning and end of the experiment. Na^{24} was assayed using a well-type crystal scintillation counter (Beckman, model 2020), and all samples were counted to at least 10,000 counts. Since the initially labeled compartment is essentially of infinite size, the unidirectional flux may be calculated as follows:—

$$\Phi_{ms}^{Na} = v_s(p_{s2} - cp_{s1})/(\Delta t p_m^* A) \quad (1)$$

where p_s = CPM/cm³ in the serosal reservoir
c = correction factor for dilution
v_s = volume of medium perfusing the serosal surface in cm³
A = area of membrane = 1.13 cm²
Δt = time interval between samples 1 and 2 in hrs.
p_m^* = specific activity of Na^{24} in the mucosal solution in CPM/μmol
Φ_{ms}^{Na} = unidirectional Na flux from mucosa to serosa in μmol/cm² hr.

In the double label experiments the procedure was similar except that both reservoirs were sampled simultaneously, and the addition of fresh medium after withdrawal of samples was omitted. Na^{24} was assayed using the well-type crystal scintillation counter with the threshold set so as to completely exclude the weaker radiations from Na^{22}. The Na^{22} was assayed 3 weeks later. Standards containing known amounts of Na^{24} and Na^{22} singly and in combination were used to assure that the assays of these isotopes did not overlap significantly.

Other Determinations

Na and K were determined using a Coleman flame photometer (model 21).

All pH measurements were made with a Beckman (model 96) pH-meter, to an accuracy of ±0.1 unit.

RESULTS AND DISCUSSION

The Transmural PD, I_{sc}, and Tissue Conductance

The transmural PD was determined in over 150 experiments and in every instance the serosal surface was positive with respect to the mucosal surface. In the presence of glucose (11 mM) the initial transmural PD averaged 9.1 ± 0.4[1] mv (36 experiments) and slowly declined to a value of 4 to 5 mv over a 60 minute period. These values are in good agreement with those reported by Clarkson *et al.* (4–6) and Curran (13) for *in vitro* preparations of rat ileum.

The initial I_{sc} in the presence of glucose (36 experiments) averaged 136 ± 6 μa. In Fig. 1 is shown a plot of the I_{sc} as a function of time in 16 experiments.

[1] All variance is expressed as standard errors of the mean (SE) unless otherwise indicated.

As in the case of the PD, the I_{sc} also declines slowly (approx. 1 μa/min.) during the experimental period. The reasons for the decline in both the PD and the I_{sc} are as yet unclear; however, they appear to be related to the coupling between active glucose and Na transport (14) and will be discussed in more detail below.

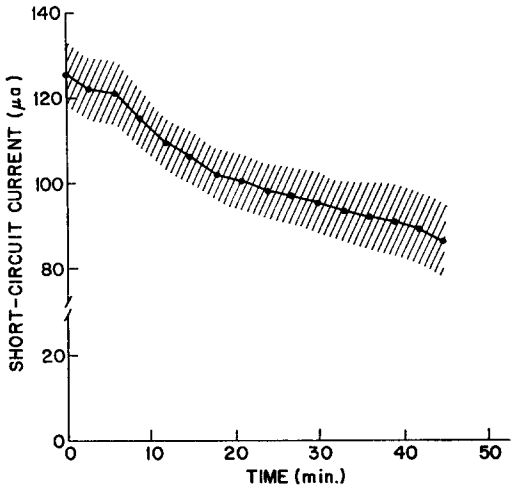

FIGURE 1. The short-circuit current, in the presence of glucose, as a function of time (16 experiments). The shaded area represents ±1 SE.

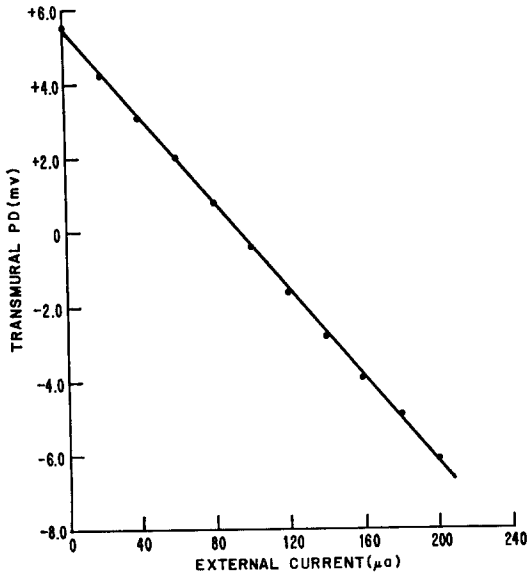

FIGURE 2. The transmural PD (uncorrected) as a function of the external current.

A plot relating the PD with the external current is shown in Fig. 2. The system behaves as a linear resistor over the range shown. Further experiments have indicated that this linear relationship is maintained over the range −30 to +50 mv (all PD's refer to the serosal surface with respect to the mucosal surface). Furthermore, at each increment of current the PD rapidly assumed a

stable level. Since the tissue behaves as a linear resistor the conductance may be calculated from the spontaneous PD and the corrected I_{sc}. The initial conductance in the presence of glucose was 14.9 ± 0.1 mmhos/cm² (36 experiments). After a 60 minute period the conductance increased 20 per cent. Thus the gradual decrease in the PD may be attributed in part to a decrease in the tissue resistance with time.

Na Fluxes

The average mucosa to serosa, Φ_{ms}^{Na}, and serosa to mucosa, Φ_{sm}^{Na}, fluxes of Na determined in both single and double label experiments in the presence of

TABLE I

COMPARISON OF NET NA FLUXES AND SHORT-CIRCUIT CURRENT IN ISOLATED RABBIT ILEUM*

Φ_{ms}^{Na}	Φ_{sm}^{Na}	Φ_{net}^{Na}	I_{sc}
	μmol/cm² hr.		
	Glucose present		
9.6±0.3 (39)	5.7±0.2 (33)	3.9±0.4	3.7±0.1 (72)
	Glucose present (double label)‡		
8.8±0.5 (21)	5.7±0.3 (21)	3.1±0.6	3.1±0.2 (21)
	Glucose absent		
9.5±0.2 (42)	6.7±0.3 (33)	2.8±0.4	2.6±0.1 (75)
	DNP (2×10^{-4} *M*)		
7.2±0.3 (6)	7.7±0.3 (6)	−0.5±0.4	0.0±0 (12)
	Ouabain (2×10^{-4} *M*)		
6.2±0.2 (13)	6.4±0.2 (13)	−0.2±0.3	0.2±0.1 (26)

* The number of observations is indicated in parentheses.

‡ In the double label experiments glucose was added to the reservoirs at 20 minutes and sampling was started at 35 minutes. The diminished Φ_{net}^{Na} and I_{sc} may be attributed to the age of the preparation, as discussed in the text.

glucose are given in Table I. The ratio of the unidirectional fluxes of Na is in good agreement with those reported by Curran (13) and Green *et al.* (15) for the isolated small intestine of rat. The net Na flux, Φ_{net}^{Na}, from mucosa to serosa, agrees well with the average I_{sc} expressed in units of flux of a monovalent cation. Furthermore, as mentioned previously, the use of the uncorrected I_{sc} does not significantly affect this conclusion. The good agreement between Φ_{net}^{Na} and the uncorrected I_{sc} is in part due to the fact that the net Na flux from mucosa to serosa varies inversely with the transmural PD; that is, as the serosal surface becomes more positive, the mucosa to serosa net flux decreases (see Fig. 6). Thus, when the potentiometer reads zero, not only is the measured current too low, but, because a small PD (equal to approxi-

mately 13 per cent of the spontaneous PD) persists across the membrane, the Φ^{Na} is also lower than it would be in the truly short-circuited state. Furthermore, as will be seen below, these errors are of similar magnitude.

From the data presented in Table I and the above considerations one may conclude that most if not all of the true I_{sc} can be accounted for by the net transport of Na from the mucosal to the serosal solutions in the absence of an electrochemical potential gradient. The agreement between Φ^{Na}_{net} and I_{sc} cannot be established with confidence, in the present experiments, to better than ± 15 per cent because of the high Φ^{Na}_{sm} and the experimental errors inherent in the flux measurements.

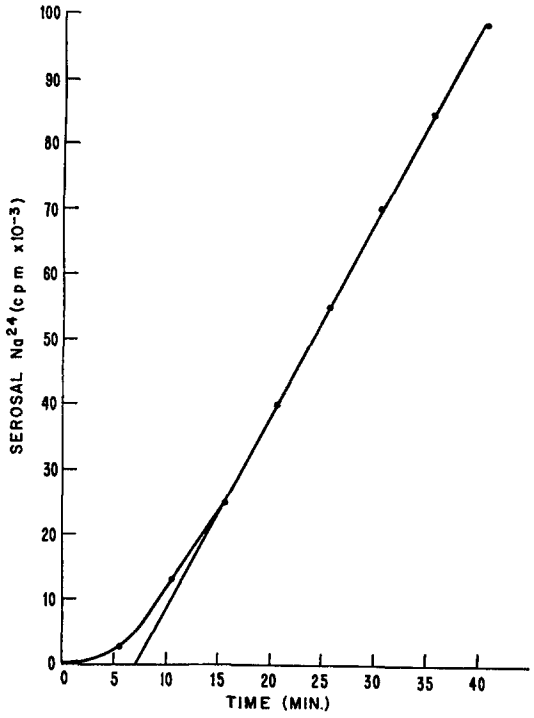

FIGURE 3. The total serosal Na^{24} activity as a function of time after the addition of the tracer to the mucosal reservoir.

In Fig. 3 we have plotted the total Na^{24} in the serosal solution as a function of time after the addition of the tracer to the mucosal solution. The curve, typical of that found in 6 such experiments, indicates that a steady-state flux of Na^{24} is not achieved until after 15 to 20 minutes have elapsed. As discussed by Hoshiko and Ussing (16) and Diamond (17), two factors contribute to this delay. The first is the time necessary for the intracellular Na to achieve a steady-state specific activity and, under these circumstances, the approach to a steady-state transmural flux should be an exponential function of time and related to the intracellular Na pool size. The second is the time necessary to reach a steady-state rate of diffusion of Na^{24} through the tissue. In this case,

the approach to a steady-state transmural Na^{24} flux should consist of an infinite series of exponential functions of time, and it can be shown (18) that the time-intercept (t) of the line tangent to the steady-state slope is related to the diffusion coefficient (D') of Na in the tissue by

$$t = \lambda^2/6D' \qquad (2)$$

where λ is the thickness of the diffusion barrier. Assuming that only this factor is responsible for the observed time course, and using a value of 0.5 mm for λ, the average value for D' is 0.8×10^{-6} cm²/sec.; a value which is approximately 5 per cent of the diffusion coefficient of NaCl in water at 37°C (1.7×10^{-5} cm²/sec.). Similar values reflecting the retardation of diffusion through extracellular tissue have been reported by Page (19), Diamond (17), and others (20, 21) and may be attributed to both fixed charge effects and steric barriers. A semilogarithmic plot of the approach to the steady-state flux, after the method of Hoshiko and Ussing, could not be fit by a straight line, indicating that the intracellular Na pool is not the predominant factor in the delay. However, since it must contribute to some degree, the value of D' must be considered a minimal value.

Effect of Glucose-Free Media

When the tissue was perfused with the basic electrolyte medium from which glucose was omitted the initial transmural PD averaged 5.4 ± 0.1 mv (114 experiments) and the initial I_{sc} averaged 83 ± 1 μa; both values are significantly different from those observed in the presence of glucose. On the other hand, the tissue conductance averaged 15.3 ± 0.1 mmhos/cm², a value which is in good agreement with that observed in the presence of glucose. The unidirectional and net fluxes of Na and the average simultaneous I_{sc} measured in the absence of glucose are given in Table I. The Φ_{net}^{Na} is approximately 70 per cent of that found in the presence of glucose, and is in good agreement with the average I_{sc}.

In contrast to the declining I_{sc} observed in the presence of glucose, the I_{sc} and Φ_{net}^{Na} in the absence of glucose remained essentially constant for periods exceeding 1 hour. The ability of the intestine to maintain this energy-dependent process in the absence of external glucose may be attributed to the metabolism of endogenous nutrient stores which has been described by Newey and coworkers (22). The observation that endogenous metabolism is capable of maintaining active Na transport at a constant level for prolonged periods, whereas the initial PD and I_{sc} in the absence of glucose are considerably lower than those in its presence, suggests a role for external glucose in Na transport other than simply acting as a source of metabolic energy. We have demonstrated that the addition of the actively transported non-metabolized glucose

analogue, 3-0-methylglucose to a non-glucose–containing medium results in a rapid increase in the I_{sc} similar in magnitude to that observed if glucose itself is added (14). The addition of fructose, which is not actively transported but is readily metabolized, to a non-glucose medium, has no effect on the I_{sc}. These observations indicate that the increased I_{sc} observed in the presence of glucose is due to the stimulation of Na transport by the active transport of the sugar *per se*. Subsequent experiments have shown that the increase in the I_{sc} resulting from the addition of an actively transported sugar, metabolized or not, to a glucose-free medium diminishes with time. Thus the gradual decrease in the I_{sc} observed in the presence of glucose, but not in a glucose-free medium,

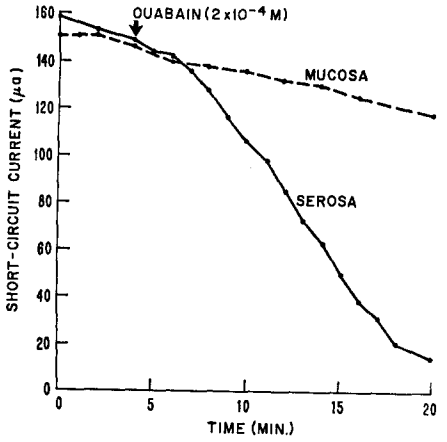

FIGURE 4. The effect of ouabain (2×10^{-4} M) following addition to either the mucosal or serosal solutions. The two tissues were adjacent portions of distal ileum obtained from the same animal.

may be attributed to a gradual decrease in the interaction between active sugar and Na transport.

Effect of Metabolic Inhibitors

The I_{sc} is rapidly abolished by anaerobiosis, KCN (10^{-3} M), and 2,4-dinitrophenol (DNP). In Table I are shown the unidirectional and net Na fluxes, and the average I_{sc} in the presence of 2×10^{-4} M DNP. These results indicate that active Na transport by the rabbit intestine is dependent upon an intact aerobic metabolic pathway. Furthermore, NaF (10^{-3} M), *N*-ethylmaleimide (10^{-3} M), and the vitamin B_6 analog, mercaptopyridoxine, also rapidly abolish the I_{sc}.

The effects of both DNP and *N*-ethylmaleimide are most rapid and pronounced when these agents are added to the mucosal solutions. When added only to the serosal solution the onset of inhibition is markedly delayed and the rate of decline of the I_{sc} is significantly diminished. This may be due to the barrier presented by the serosal tissues which retards access to the transporting cell.

Effect of Ouabain

The inhibitory effect of low concentrations of the cardiac glycosides on active transport mechanisms in a wide variety of tissues has been well documented. In Fig. 4 is shown the effect of ouabain (2×10^{-4} M) on the I_{sc} in isolated rabbit ileum. This plot is typical of 6 experiments in which two adjacent portions of ileum from the same animal were examined simultaneously as described under Methods. In all instances the addition of ouabain to the serosal solution resulted in a rapid decline of the I_{sc} following a brief lag period. On the other hand, when ouabain was added to the mucosal solution either no effect or a very much diminished inhibition of the I_{sc} was observed. Hendley and Smyth (23) have reported that the intestinal Na transport mechanism in the rat is insensitive to the cardiac glycosides. Since, in their experiments, digoxin was added only to the mucosal solution, this conclusion must be reevaluated.

The fact that ouabain is most effective only when present in the serosal solution, despite the presence of connective tissue and muscle layers between the serosal surface and the epithelial cells, may be due to a permeability of the basal membrane of the cell to this agent greater than that of the luminal membrane. However, Caldwell and Keynes (24) have shown that ouabain inhibits Na transport only if applied to the exterior of the squid axon, microinjection into the axoplasm being ineffective. This observation coupled with our results with DNP, which must gain access to the cell interior to exert its action, suggests that the ouabain-sensitive site is situated on or near the basal membrane of the epithelial cell. Analogous locations for the Na transport mechanism have been postulated for the frog skin (25), toad bladder (26), and renal tubule (27).

In Table I are shown the unidirectional and net Na fluxes in a series of experiments in which Na^{24} was added to either the serosal or mucosal solutions after ouabain had virtually abolished the I_{sc}.

Effect of Temperature

The effect of temperature was evaluated in 4 experiments by clamping the tubing connecting the circulating pump to one set of water-jackets. In this way adjacent portions of ileum from the same rabbit were compared, one at 38.5°C, and the other at 22.4°C. The apparent activation energy of the I_{sc} in the absence of glucose was 7,300 cal/mol ($Q_{10} = 1.6$). In the presence of glucose the apparent activation energy averaged 13,600 cal/mol ($Q_{10} = 2.0$). This increase in the apparent activation energy and Q_{10} may be attributed to the fact that a large fraction of the I_{sc} in the presence of glucose is the result of the interaction between two active transport systems. The apparent acti-

vation energy of the tissue conductance was 4,200 cal/mol as would be expected if this property were primarily determined by free diffusion forces.

At the completion of these experiments the circulation from the constant temperature pump to the room temperature water-jackets was reestablished. This resulted in a rapid rise in the I_{sc} reaching a value which did not differ significantly from the control (38.5°C), indicating that the temperature effect is completely reversible and that the two tissues were indeed comparable.

Effect of Na *Concentration on* I_{sc}

The results of a series of experiments designed to evaluate the relationship between the I_{sc} and the Na concentration in the medium, in the absence of

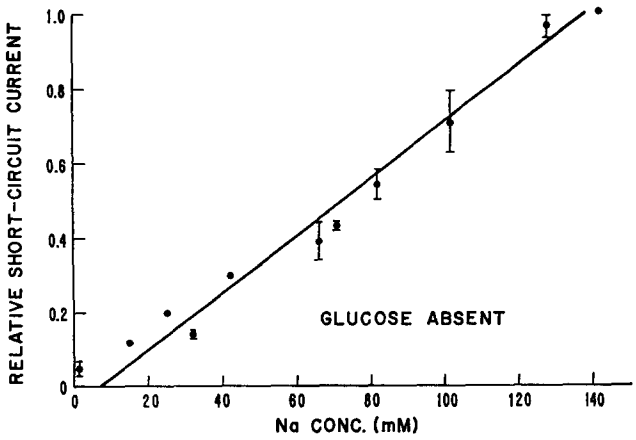

FIGURE 5. The effect of the Na concentration in the bathing medium on the short-circuit current. The line was obtained by the method of least-squares. The average initial I_{sc} (Na = 142 mM) was 89 μa.

glucose, are shown in Fig. 5. In these experiments (except those in which the Na concentration was 1.1 mM) the initial medium contained 142 mM Na. The Na concentration was then lowered by serial dilution of the solutions in both reservoirs using a medium in which NaCl was replaced by KCl. After each dilution the I_{sc} declined and was allowed to achieve a fairly stable level prior the the next dilution.

In 4 experiments the tissue was initially perfused with the Na-free medium. After 30 minutes the Na concentration averaged 1.1 mM, and in each instance the PD and I_{sc} were slightly greater than zero.

The use of KCl in these experiments as a substitute for NaCl was dictated by several factors. First, the specific resistance of the K medium is 20 per cent less than that of the normal medium, thus during the course of the dilutions the error in the I_{sc} due to the fluid resistance will vary from 13 per cent initially,

to 10 per cent in the Na-free medium. Second, several experiments using choline-Cl as the substitute for NaCl were unsatisfactory because of the occurrence of marked transients in both the PD and I_{sc}. After the addition of the choline medium to both reservoirs the I_{sc} and PD fell rapidly to low levels and then slowly rose to levels somewhat lower than those which would have been obtained with the K medium. Kirschner has recently demonstrated that the passive movements of Na and choline across frog skin are of similar magnitude (28). Renkin has also shown that choline enters the cells of frog skeletal muscle at a rate similar to that of Na, but that its exit rate is considerably slower presumably because of the high selectivity of the "Na pump" (29). Since the rabbit ileum is highly permeable to Na, choline might be expected to enter the cell rapidly under the influence of a large concentration gradient, and result in an alteration of the intracellular cation profile. If the serosal surface of the cell is more permeable to choline than the mucosal surface, the negative transients may be attributed to the passive movements of this cation.

As shown in Fig. 5, the relationship between the I_{sc} and the Na concentration of the medium is linear over the range studied, and, in the absence of Na the I_{sc} does not differ significantly from zero. These results support the flux data in ascribing the I_{sc} to net Na transport, and, since the Cl concentration was constant, indicate that a significant independent Cl current does not exist. A linear relationship between the Na concentration of the medium and net Na transport has been observed in rat colon (30). However, saturation kinetics are observed in most other systems which have been investigated (3, 17, 25, 26). We have shown that in the presence of actively transported sugars and amino acids (31) the I_{sc} may reach levels as high as 240 μa when the Na concentration is 142 mM. The linear relationship observed up to an average I_{sc} of 89 μa in the present experiments, may be explained by assuming that the entrance of Na into the cell across the mucosal surface is rate-limiting, and that the carrier mechanism is operating at a level far from saturation.

Effect of K-Free Medium on I_{sc}

The effect of a K-free medium on the I_{sc} was evaluated in 4 experiments. In each instance the experimental tissue was compared with a control tissue which was perfused with the identical medium but contained 5 mM K. Observations were carried out over periods exceeding 1 hour. At the end of this time the medium was found to contain approximately 0.3 mM K, which may be attributed to leakage from the tissue. The I_{sc} and PD in the absence of K did not differ significantly from the control values. Furthermore, the addition of 5 mM K to the K-free medium was without significant effect.

In both the toad bladder (32) and the frog skin (25) removal of K from the bathing medium essentially abolishes the I_{sc} after a variable lag period. Dia-

mond has reported that removal of K from the medium bathing the serosal surface of the fish gall bladder results in a 50 per cent decrease in the net Na transport after a lag of about 30 minutes (17). Essig and Leaf (33) have recently suggested that the effect of K removal in the toad bladder may be attributed to a decrease in the permeability of the mucosal barrier to Na rather than to a 1 for 1 coupling between K and Na movements as had been previously postulated (25). In the present experiments the absence of a K effect may be the result of either a very prolonged lag, which exceeds our period of observation, or the fact that the medium was not strictly "K-free." Barring these reservations however, the present results suggest that active Na transport in rabbit ileum is not necessarily coupled to K transport, and that, as in the case of toad bladder, an electrogenic mechanism may be operative.

Active and Passive Components of Na *Fluxes*

As shown in Table I, Φ_{sm}^{Na} remains relatively constant under a variety of experimental conditions, with the exception of the experiments employing DNP. In the latter, the increased Φ_{sm}^{Na} may be attributed to an increase in the tissue permeability resulting from prolonged metabolic inhibition. In a total of 121 observations Φ_{sm}^{Na} averaged 6.1 $\pm$ 0.2 μmol/cm^2 hr., and was not significantly influenced by the presence of sugars, amino acids, or ouabain. These results suggest that the unidirectional serosa to mucosa flux is the result of the passive diffusion of Na. However, in the absence of further evidence one can neither rule out the presence of a carrier-mediated exchange diffusion component nor evaluate the influence of solvent-drag forces on the flux.

Ussing and others have demonstrated both in living (34) and artificial permselective (35) membranes that passive ionic fluxes, ${}_p\Phi^i$, in the absence of exchange diffusion, solvent drag, or single file diffusion are related by

$$ {}_p\Phi_{12}^i / {}_p\Phi_{21}^i = (a_1^i / a_2^i) \exp [z_i F (\psi_1 - \psi_2)/RT] \quad (3) $$

where a = ionic activity, ψ = electrical potential, subscripts 1 and 2 denote the two compartments, and z, F, R, and T have their usual meanings.

The partial conductance, G_i, of a passively transported ion (as defined by Equation 3) when the ionic activities on both sides of the membrane are equal, is given by (36)

$$ G_i = {}_p\Phi_{12}^i F \{\exp [z_i F(\psi_2 - \psi_1)/RT] - 1\}/(\psi_2 - \psi_1) \quad (4) $$

and, when $\psi_2 - \psi_1 = 0$, Equation 4 reduces to

$$ {}_oG_i = {}_{po}\Phi_{12}^i z_i^2 F^2/RT \quad (5) $$

where the subscript o refers to the short-circuit condition.

Equations 4 and 5 cannot be used to calculate the partial conductance of Na in the present experiments because of the presence of active transport. However, they may be used to describe the effect of the transmural PD on Φ_{sm}^{Na} if this flux is passive, and if the passive permeability of the tissue is not a function of the transmural PD. Under the latter conditions, a change in the transmural PD should result in a predictable change in Φ_{sm}^{Na}. For this purpose we define ${}_pG_{Na}$ as the partial Na conductance which would result if Na transport were purely passive, as, for example, under conditions in which active Na transport is inhibited by ouabain.

Assuming that Φ_{sm}^{Na} is passive and that ${}_pG_{Na}$ is independent of the transmural PD we may combine Equations 4 and 5 with the appropriate substitutions to obtain

$$\Phi_{sm}^{Na}/{}_o\Phi_{sm}^{Na} = \frac{F(\psi_m - \psi_s)/RT}{\exp[F(\psi_m - \psi_s)/RT] - 1} \tag{6}$$

The expression on the right-hand side of Equation 6 is of the form $x/(e^x - 1)$ which when expanded can be shown to approximate $e^{-x/2}$, thus

$$\frac{F(\psi_m - \psi_s)/RT}{\exp[F(\psi_m - \psi_s)/RT] - 1} \doteq \{\exp[F(\psi_s - \psi_m)/RT]\}^{1/2} \tag{7}$$

The error of this approximation is less than 6 per cent over the range of 25 to −25 mv, and is 13 per cent when the PD is ±50 mv. Using the value 6.1 μmol/cm² hr. for ${}_o\Phi_{sm}^{Na}$ we obtain

$$\Phi_{sm}^{Na} \doteq 6.1\{\exp[F(\psi_s - \psi_m)/RT]\}^{1/2} \tag{8}$$

and, combining Equations 3 and 8

$${}_p\Phi_{ms}^{Na} \doteq 6.1\{\exp[F(\psi_s - \psi_m)/RT]\}^{-1/2} \tag{9}$$

where ${}_p\Phi_{ms}^{Na}$ is the passive component of the mucosa to serosa Na flux.

In Fig. 6 (upper) we have plotted the values of Φ_{sm}^{Na} determined using Na^{24}, when the tissue was maintained at PD's varying from −30 to 50 mv. The solid line was drawn from Equation 8. The good agreement between Φ_{sm}^{Na} and Equation 8 suggests that if the assumptions leading to Equation 6 are valid, the serosa to mucosa Na flux is consistent with passive diffusion uncomplicated by exchange diffusion, single file diffusion, or solvent drag.

This conclusion is supported by the observation that, assuming Φ_{sm}^{Na} to be passive, the values of ${}_pG_{Na}$ at the different PD's calculated from Equation 4, did not differ significantly and averaged 6.1 ± 0.2 mmhos/cm², a value which is essentially equal to ${}_{po}G_{Na}$ as given by Equation 5.

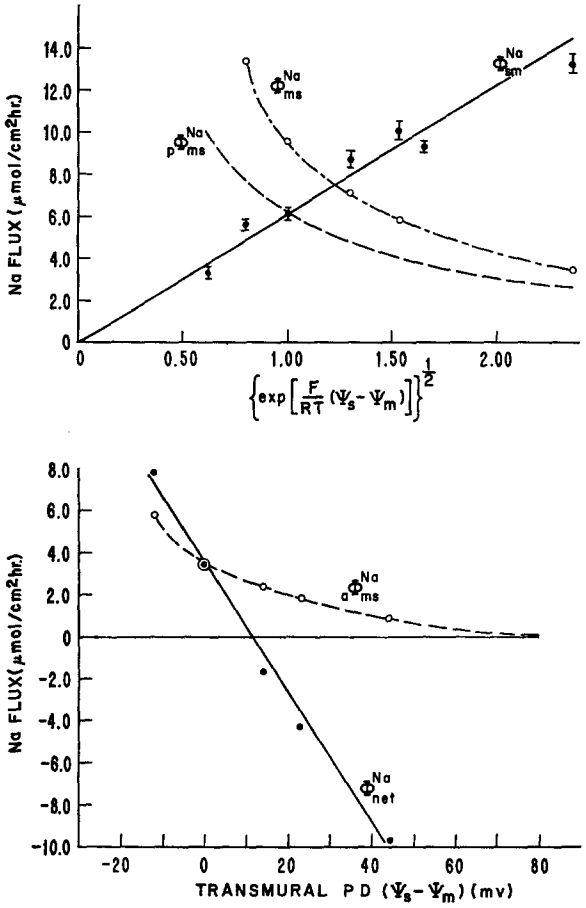

FIGURE 6. Upper, Φ^{Na}_{sm} (closed circles), Φ^{Na}_{ms} (open circles), and $_p\Phi^{Na}_{ms}$ as functions of the transmural PD. Lower, $_a\Phi^{Na}_{ms}$ (open circles), and Φ^{Na}_{net} (closed circles) as functions of the transmural PD. A positive Na flux indicates movement from mucosa to serosa; a negative value indicates the reverse movement. The transmural PD's were determined from the total PD after correcting for fluid resistance.

The present formulation leading to Equations 8 and 9[2] enables one to determine readily whether a unidirectional ionic flux is the result of passive diffusion alone, in the presence of active transport of that ion in the opposite direction, providing that it can be demonstrated that the passive resistance to the movement of the ion in question is not a function of the PD.

Also shown in the upper portion of Fig. 6 are the values of Φ^{Na}_{ms} determined

[2] The relationship between Φ^{Na}_{sm} and $_o\Phi^{Na}_{sm}$ is given by Equation 6. The value of the approximation leading to Equations 8 and 9 is that it simplifies the expression and calculation of Φ^{Na}_{sm} and $_p\Phi^{Na}_{ms}$ while introducing a relatively small error over a wide range of PD's.

at various PD's in the presence of glucose, and the values of ${}_p\Phi_{ms}^{Na}$ calculated from Equation 9. The difference between Φ_{ms}^{Na} and ${}_p\Phi_{ms}^{Na}$ represents the active component of Na transport, ${}_a\Phi_{ms}^{Na}$, which is plotted as a function of PD in the lower portion of Fig. 6. Also shown in this lower figure are the values of Φ_{net}^{Na} at the different PD's. Ussing and Zerahn have defined the active transport potential of Na, E_{Na}, as the transmural PD at which net Na transport is abolished (9). As can be seen in Fig. 6 (lower) the E_{Na} for rabbit ileum is approximately 11.6 mv. The E_{Na} may also be defined as the electrical potential difference which must be superimposed upon the electrochemical potential difference in order that the measured fluxes satisfy Equation 3, and may be calculated from (36)

$$\Phi_{sm}^{Na}/\Phi_{ms}^{Na} = (a_s/a_m) \exp [F (\psi_s - \psi_m - E_{Na})/RT] \quad (10)$$

Using the Na fluxes measured under the short-circuited condition E_{Na} is calculated to be 12.2 mv, in good agreement with that obtained above.

It is interesting to compare the E_{Na} of rabbit ileum with that determined by Ussing and Zerahn for frog skin. In the latter case the E_{Na} determined by the methods described above averages 120 mv and is largely a measure of the effect of the skin PD on the active transport mechanism. Because of the low Na permeability of frog skin, changes in the passive flux with varying PD's contribute only slightly to the change in net Na transport (see Fig. 2, reference 9). In the present experiments, the fact that Φ_{net}^{Na} is abolished at a PD of 12 mv is largely due to the high tissue Na permeability. Since the mucosa of the ileum does not consist of a single cell type, this permeability may be attributed not only to the cells responsible for Na transport but to the intercellular spaces and other cells as well. The apparent E_{Na}, then, is a measure of the over-all efficiency of the tissue with respect to net Na transport but cannot be applied to the transporting cell itself. As shown in the lower portion of Fig. 6, if the cell responsible for Na transport were essentially impermeable to Na, the E_{Na} of the cell would be in the range of 60 to 80 mv.

Using the value of 6.1 μmol/cm^{2}hr. as the steady-state rate of diffusion of Na across the tissue the calculated diffusion coefficient of Na in the tissue is 0.6×10^{-6} cm^2/sec., in good agreement with the value for D' (0.8×10^{-6} cm^2/sec.) obtained by extrapolation of the Na^{24} data.

CONCLUSIONS

The present results are similar to those which have been reported for the frog skin (9), toad bladder (10), the large intestine of the toad (11) and frog (12), and the cecum of the guinea pig (11) in that most if not all of the current generated by the short-circuited *in vitro* preparation may be ascribed to the active transport of Na. Although the present experiments do not rule out the possibility that Cl is actively transported by the *in vitro* rabbit ileum, they

do indicate that either: (*a*) The Cl current in the short-circuited state is small compared with the Na current (less than 15 per cent); or (*b*) that it is almost exactly masked under a variety of experimental conditions by either the active transport of another anion in the opposite direction or the active transport of a cation, other than Na, in the same direction.

The views expressed herein are those of the authors and do not necessarily reflect the views of the United States Air Force or the Department of Defense.

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BIBLIOGRAPHY

1. DURBIN, R. P., CURRAN, P. F., and SOLOMON, A. K., *Adv. Biol. and Med. Physics*, 1959, **6**, 1.
2. WILSON, T. H., Intestinal Absorption, Philadelphia, W. B. Saunders Company, 1962.
3. CURRAN, P. F., and SOLOMON, A. K., *J. Gen. Physiol.*, 1957, **41**, 143.
4. CLARKSON, T. W., and ROTHSTEIN, A., *Am. J. Physiol.*, 1960, **199**, 898.
5. CLARKSON, T. W., ROTHSTEIN, A., and CROSS, A., *Am. J. Physiol.*, 1961, **200**, 781.
6. CLARKSON, T. W., CROSS, A. C., and TOOLE, S. R., *Am. J. Physiol.*, 1961, **200**, 1233.
7. VAUGHAN, B. E., and ALPERN, E. L., U. S. Naval Radiological Defense Laboratory Report TR-405, March, 1960.
8. SMYTH, D. H., and TAYLOR, C. B., *J. Physiol.*, 1957, **136**, 632.
9. USSING, H. H., and ZERAHN, K., *Acta Physiol. Scand.*, 1951, **23**, 110.
10. LEAF, A., ANDERSON, J., and PAGE, L. B., *J. Gen. Physiol.*, 1958, **41**, 657.
11. USSING, H. H., and ANDERSON, B., *Proc. 3rd Internat. Cong. Biochem.*, Brussels, 1955, 434.
12. COOPERSTEIN, I. L., and HOGBEN, C. A. M., *J. Gen. Physiol.*, 1959, **42**, 461.
13. CURRAN, P. F., *J. Gen. Physiol.*, 1960, **43**, 1137.
14. SCHULTZ, S. G., and ZALUSKY, R., *Biochim. et Biophysica Acta*, 1963, **71**, 503.
15. GREEN, K., SESHADRI, B., and MATTY, A. J., *Nature*, 1962, **196**, 1322.
16. HOSHIKO, T., and USSING, H. H., *Acta Physiol. Scand.*, 1960, **49**, 74.
17. DIAMOND, J. M., *J. Physiol.*, 1962, **161**, 474.
18. CRANK, J., The Mathematics of Diffusion, Oxford, Clarendon Press, 1956, 48.
19. PAGE, E., *Abstr. 7th Ann. Meeting Biophysic. Soc.*, 1963, WC9.
20. COTLOVE, E., *Am. J. Physiol.*, 1954, **176**, 396.
21. KEYNES, R. D., *Proc. Roy. Soc. London, Series B*, 1954, **142**, 359.
22. NEWEY, H., PARSONS, B. J., and SMYTH, D. H., *J. Physiol.*, 1959, **148**, 83.
23. HENDLEY, E., and SMYTH, D. H., *J. Physiol.*, 1957, **139**, 27P.

24. CALDWELL, P. C., and KEYNES, R. D., *J. Physiol.*, 1959, **148,** 8P.
25. USSING, H. H., *in* The Alkali Metal Ions in Biology, Berlin, Springer Verlag, 1960.
26. FRAZIER, H. S., DEMPSEY, E. F., and LEAF, A., *J. Gen. Physiol.*, 1962, **45,** 529.
27. WHITTEMBURY, G., SUGINO, N., and SOLOMON, A. K., *J. Gen. Physiol.*, 1961, **44,** 689.
28. KIRSCHNER, L. B., *Science,* 1960, **132,** 85.
29. RENKIN, E. M., *J. Gen. Physiol.*, 1961, **44,** 1159.
30. CURRAN, P. F., and SCHWARTZ G. F., *J. Gen. Physiol.*, 1960, **43,** 555.
31. ZALUSKY, R., and SCHULTZ, S. G., *Clin. Research,* 1963, **11,** 189.
32. HAYS, R. M., and LEAF, A., *Ann. Int. Med.*, 1961, **54,** 700.
33. ESSIG, A., and LEAF, A., *J. Gen. Physiol.*, 1963, **46,** 505.
34. USSING, H. H., *Acta Physiol. Scand.*, 1949, **19,** 43.
35. MEARES, P., and USSING, H. H., *Tr. Faraday Soc.*, 1959, **55,** 142.
36. LINDERHOLM, H., *Acta Physiol. Scand.*, 1952, **27,** suppl. 97, 1.