The Transport of Salt and Water across Isolated Rat Ileum

Evidence for at least two distinct pathways

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ABSTRACT The flows of sodium, potassium, and chloride under electrical and chemical gradients and of salt and water in the presence of osmotic pressure gradients are described by phenomenological equations based on the thermodynamics of irreversible processes. The aim was to give the simplest possible description, that is to postulate the least number of active transport processes and the least number of separate pathways across the intestine. On this basis, the results were consistent with the following picture of the intestine: Two channels exist across this tissue, one allowing only passive transport of ions and the other only active. In the passive channel, the predominant resistance to ion flow is friction with the water in the channel. The electroosmotic flow indicates that the passive channel is lined with negative fixed charged groups having a surface charge density of 3000 esu cm^{-2}. The values of the ion-water frictional coefficients, and the relationship between ionic concentrations and flows indicate that the passive channel is extracellular. The active channel behaves as two membranes in series, the first membrane being semipermeable but allowing active transport of sodium, and the second membrane being similar to the passive channel. Friction with the ions in the second "membrane" is the predominant resistance to water flow.

INTRODUCTION

Published reports indicate a strong interdependence between the flows of salts (or solutes generally) and water across the ileum. One point of view, first put forward by Ingraham and Visscher (1) and recently reaffirmed by Grim (2) for transport across gall bladder, maintains that salts are transported by entrainment in the water flow. The other point of view postulates that water flow is dependent on solute flow (Curran and Solomon (3); Clarkson and Rothstein, (4)) and that water may be transported against its activity gradient by active transport of solutes (Curran, 5).

This work was prompted by the "friction model" as elucidated by Kedem and Katchalsky (6) which takes into account frictional interaction between the flows of different species. A similar model has been successfully applied to ion exchange membranes (7). Recently Kedem (8) has demonstrated that this model offers a simple method for quantitative treatment of water flow in the presence of active transport of solutes.

The plan of this paper is first to report ion and water flows under controlled electrical and chemical potential gradients, and second to describe quantitatively the flows by a friction model invoking the least number of active transport processes and the least number of separate pathways across the ileum.

Previous observations on this tissue indicate the presence of at least one active transport process (3, 4) and at least two distinct types of pathways across the ileum. Two pathways are indicated by both functional and morphological observations. In vivo measurements on the diffusion of mannitol across the ileum (3) indicate the presence of aqueous pores having diameters of the order of 30 to 40 A whereas the equivalent mean pore radius of the membrane of the epithelial cell is only 4 A (9). Morphologically the epithelial cell layer is penetrated by extracellular channels, albeit transient ones. Epithelial cells are produced in the crypts of Lieberkühn, and move along the tissue surface to the tips of the villi where they exfoliate into the lumen (10). The gaps left by the exfoliation of cells have been seen in the microscope (11). It would appear that we may expect to find at least two pathways across the ileum, one having a large diameter, located extracellularly and allowing passive transport of salt and water, the other channel having a small diameter, located intracellularly at least through part of its length, and associated with active transport processes. This "literature" model of the intestine will be expressed more formally in terms of the phenomenological equations of irreversible thermodynamics as expressed by the friction model of Kedem and Katchalsky.

A FRICTION MODEL BASED ON THE LITERATURE

The equation of flow of ions and water through the passive channel will first be derived following the general treatment of Kedem and Katchalsky (6). The passive channel is assumed to be continuous across the ileum, from mucosal to serosal sides, to be aqueous and of large diameter compared to the diameter of sodium, chloride, and water. The flows of the ith ion J_i and of water J_w, positive when in the mucosal to serosal direction, are driven by forces X_i and X_w respectively, where X_i is the gradient of the electrochemical potential of the ion i, $-d\bar{\mu}_i/dx$, and X_w the chemical potential gradient of water, $-d\mu_w/dx$. The thermodynamic driving forces operating on each ionic species and on water are balanced by a linear superposition of frictional forces during steady-state flow. In general friction may arise between (1) ions or

water and the membrane matrix, (2) water and ions, and (3) different ions (7). In this treatment it will be assumed that the aqueous channel is so wide that frictions with the membrane matrix are insignificant. Furthermore friction between the ions will be ignored so that the only frictional force regarded as significant is that between the ions and water. Thus for the ith ion,

$$x_i = -d\bar{\mu}_i/dx = -F_{iw} \tag{1}$$

where F_{iw} is the mechanical friction of the ion i with water. Similarly, the thermodynamic force on water is given by the sum of the frictions with each ion

$$X_w = d\mu_w/dx = \sum_i F_{wi} \tag{2}$$

According to hydrodynamic convention, the frictional forces F_{ij} are proportional to the relative velocity of the ith vs. the jth component $(v_i - v_j)$ or

$$F_{ij} = -f_{ij}(v_i - v_j) \tag{3}$$

the proportionality factor f_{ij} being the coefficient per mole of the ith component (7).

If the velocities of ion and water at any point x in the passive channel, (x being the distance normal to the mucosal surface), are v_i and v_w respectively, the corresponding flows will be

$$J_i = C_i v_i = dn_i/dt \quad \text{and} \quad C_w v_w = dn_w/dt \tag{4}$$

where dn_i/dt is the number of moles of ion, i, passing through unit area of the passive channel per unit time and C_i is the molar concentration at point x. The total flows of ion and water, J_i^P and J_w^P, through the passive channel will be related to flows per unit area by the equation

$$J_i^P/A^P = J_i \quad \text{and} \quad J_w^P/A^P = J_w \tag{5}$$

where A^P is the total area of all passive channels across the ileum. Equations (1 to 5) lead to the following expressions for ion and water flows:

$$(-d\bar{\mu}_i/dx) = (f_{iw}/A^P)(J_i^P/C_i^P - J_w^P/C_w^P) \tag{6}$$

$$(-d\mu_w/dx) = \sum_i (f_{wi}/A^P)(J_w^P/C_w^P - J_i^P/C_w^P) \tag{7}$$

Reasoning from Onsager's law of reciprocal relations, Kedem and Katchalsky (6) have shown that the strongly concentration-dependent coefficient, f_{wi}, may be replaced by the approximately concentration-independent coefficient, f_{iw}, by the expression

$$f_{wi}/C_i = f_{iw}/C_w \tag{8}$$

and substitution for f_{wi} in equation (7) leads to

$$-C_w^P(d\mu_w/dx) = \sum_i (C_i^P f_{iw}/A^P)(J_w^P/C_w^P - J_i^P/C_i^P) \tag{9}$$

Equations (6) and (9) must be integrated across the length of the passive channel, Δx^P, to provide measurable magnitudes. The left-hand side of equation (6) may be integrated directly, assuming that the solution at each end of the channel is in equilibrium with the bathing solutions. To effect the integration of the right-hand side of equation (6), it will be assumed that the frictional coefficients, f_{iw}, the area, A^P, and following Kirkwood (12), the flows, are independent of x when in the stationary state. C_i^P is a function of x only. Moreover, C_i^P may be substituted by its corresponding value, c_i, in the external equilibrium solution by the relation given by Kedem and Katchalsky (6).

$$C_i^P/c_i = k_i \tag{10}$$

where k_i is the distribution coefficient of the ion i. In this treatment, k_i will be taken as very close to unity as would be expected for a wide aqueous channel of low fixed charge density. Thus, on integration across the passive channel, equation (6) becomes

$$\Delta\tilde{\mu}_i = f_{iw}(\Delta x^P/A^P)(J_i^P/\bar{c}_i - J_w^P/\bar{c}_w) \tag{11}$$

where $\bar{c}_i$ and $\bar{c}_w$ are the mean concentrations of ion and water in the passive channel as defined by

$$\begin{aligned} 1/\bar{c}_i &= \int_0^{x^P} \frac{dx}{C_i^P} \Big/ \Delta x^P \\ 1/\bar{c}_w &= \int_0^{\Delta x^P} \frac{dx}{C_w^P} \Big/ \Delta x^P \end{aligned} \tag{12}$$

To integrate this expression requires a knowledge of the relationship between C_i^P and x. Assuming this is linear,

$$C_i^P = ax + c_i^M \tag{13}$$

where $a = (c_i^S - c_i^M)/\Delta x^P$, and the integrated form of equation (12) is

$$\bar{c}_i = \Delta c_i/\ln c_i^M/c_i^S \tag{14}$$

where $\Delta c_i = a\Delta x^P$, and c_i^M and c_i^S are the ion concentrations in the mucosal and serosal solutions, respectively. Equation (14) defines the mean concentra-

tion in the passive channel as the logarithmic mean of the concentration of ion, i, in the mucosal and serosal solutions. The term $J_w^P/\bar{c}_w$ given in equation (11) is approximately equal to the observed volume flow for dilute solutions, so that equation (11) may be rewritten

$$\Delta\tilde{\mu}_i = f_{iw}(\Delta x^P/A^P)(J_i^P/\bar{c}_i - J_v^P) \tag{15}$$

All the variables in the right-hand side of equation (15) are directly measurable and $\Delta\tilde{\mu}_i$, expressed in millivolts, may be calculated from the ionic concentrations c_i^M and c_i^S, and the voltage $\Delta\psi$ across the intestine by the explicit thermodynamic expression

$$\Delta\tilde{\mu}_i = (RT/\mathfrak{F}) \ln c_i^M/c_i^S + z\Delta\psi \tag{16}$$

To integrate the left-hand side of equation (9), the equation derived by Kedem and Katchalsky (6) is used, that is

$$-\int_{\mu_w(o)}^{\mu_w(\Delta x^P)} C_w^P \, d\mu_w = \varphi_w^P(\Delta p - \Delta\pi_i - \Delta\pi_s) \tag{17}$$

where φ_w^P is the volume fraction of water in the passive channel membrane, Δp the hydrostatic pressure difference, and $\Delta\pi_i$ and $\Delta\pi_s$, the osmotic pressure difference of permeant and impermeant solutes, respectively. In the case of a wide aqueous channel, φ_w^P will be very close to unity and, for the experiments reported here, Δp and $\Delta\pi_i$ are zero. The left-hand side of equation (9) may be integrated on the same assumptions as in the case of equation (8) so that the integrated equation for water flow is

$$\begin{aligned} -\Delta\pi_s = \sum_i (f_{iw}/A^P) \int_o^{\Delta x^P} J_v^P C_i^P \, dx - \sum_i (f_{iw}/A^P) \int_o^{\Delta x^P} J_i^P \, dx \\ = (\Delta x^P/A^P) J_v^P \sum_i f_{iw} \bar{c}_i - (\Delta x^P/A^P) \sum_i f_{iw} J_i^P \end{aligned} \tag{18}$$

where the mean concentration in the passive channel, $\bar{c}_i$, is defined by

$$\bar{c}_i = \int_o^{\Delta x} C_i^P \, dx/\Delta x^P \tag{19}$$

If the relationship between C_i^P and x is linear, it may be shown that $\bar{c}_i$ is the arithmetic mean of the concentration in the mucosal and serosal solutions. Thus the two estimates of the mean values of $\bar{c}_i$ given by equations (12) and (19) differ depending upon whether the equation is derived for ion flow (equation 12) or for water flow (equation (19)). However, the two definitions of $\bar{c}_i$ are nearly identical if Δc_i is not too large.

The flows through the active channel are assumed to be constant and independent of the applied thermodynamic forces. The assumption is tenta-

tive and is included in this model to see whether the observed flows may be treated as the sum of two independent parallel flows across the intestine—one of which changes according to the applied thermodynamic forces while the other flow is constant. Thus the observed flows (superscript obs) across the ileum will be the sum of the flows through the active (superscript A) and passive (superscript P) channels

$$J_i^{obs} = J_i^P + J_i^A \tag{20}$$

$$J_v^{obs} = J_v^P + J_v^A \tag{21}$$

Substituting for J_i^P and J_v^P in equations (15) and (18) from equations (20) and (21) we obtain,

$$\Delta\tilde{\mu}_i = f_{iw}(\Delta x^P/A^P)(J_i^{obs}/\bar{c}_i - J_v^{obs}) - f_{iw}(\Delta x^P/A^P)(J_i^A/\bar{c}_i - J_v^A) \tag{22}$$

and

$$\begin{aligned} -\Delta\pi_s = {} & (\Delta x^P/A^P)J_v^{obs}\sum_i f_{iw}\bar{c}_i - (\Delta x^P/A^P)\sum_i f_{iw}J_i^{obs} \\ & - (\Delta x^P/A^P)J_v^A\sum_i f_{iw}\bar{c}_i + (\Delta x^P/A^P)\sum_i f_{iw}J_i^A \end{aligned} \tag{23}$$

In the absence of osmotic pressure gradients ($\Delta\pi_s = 0$) equation (23) becomes

$$J_v^{obs} = \sum_i f_{iw}J_i^{obs}/\sum_i f_{iw}\bar{c}_i + J_v^A - \sum_i f_{iw}J_i^A/\sum_i f_{iw}\bar{c}_i \tag{24}$$

In summary, the evidence in the literature, together with the simplifying assumptions made in the derivation of the "literature friction model," predicts certain relationships between the observed flows of ion and water and the thermodynamic driving forces. Equation (22) allows for any external driving force, equation (23) does not apply in the presence of hydrostatic pressure gradients, and equation (24) is restricted to experiments carried out in the absence of both hydrostatic and osmotic pressure gradients.

The experiments described in this report were designed to test the validity of these equations. Unfortunately not all the external driving forces could be tested. Even small hydrostatic pressure gradients cause serious structural changes in the isolated gut (for discussion see reference 13). Furthermore, variations in the chemical environment of the tissue, both with respect to chemical composition and osmolarity of the bathing media were restricted by the assumption that active transport processes are constant.

METHODS

The apparatus and procedure for mounting the rat ileum have been described elsewhere (13) including details on the measurement of ion and water flows, of tissue content of ions and water, and on the errors in these measurements.

When mounted on the apparatus, the everted intestine, length 8 cm, surface area 36 cm^2, is held in its natural cylindrical form. Its serosal surface is bathed by a solution (the "serosal solution") of approximately 3.5 ml and the mucosal surface by a solution (the "mucosal solution") of 1000 ml volume. The solutions were maintained at 38°C. An electrical current of uniform density may be applied to the intestine by cylindrical silver-silver chloride electrodes and the voltage drop between mucosal and serosal solutions is monitored by agar-KCl bridges leading via reversible calomel cells to a continuous recording system.

TABLE I

THE FLOWS OF SODIUM AND POTASSIUM UNDER CHEMICAL POTENTIAL GRADIENTS

Experiment group No.	Sodium			Potassium				Water	Potential difference
	J_{Na}	C^M	C^S	J_K	T_K	C^M	C^S	J_v	
	equiv/hr	*mEq*	*mEq*	*equiv/hr*	*equiv/hr*	*mEq*	*mEq*		*mv*
1	+87	144	34	−94	−12	5.5	116	+0.04	−2
2	+85	145	70	−61	−8	5.8	78	+0.06	+2
3	+57	145	100	−52	−14	5.7	52	+0.08	+4
4	+45	145	123	−23	−10	5.7	27	+0.15	+7
5	+36	114	142	+29	−10	35	9	+0.37	+7
6	−10	84	141	+51	−10	65	15	+0.13	+4
7	−28	54	133	+65	−2	96	18	+0.15	+5
8	−67	18	132	+89	−10	134	20	+0.05	+2
9				−3	−10	71	70	+0.04	+2
10				+5	−10	16	15	+0.34	+2

The figures in each row are the means of three experiments. J_i is the ion flow and J_v the volume flow positive in the mucosal to serosal direction. T_K is the charge in tissue content of potassium, positive when the tissue content increases. c^M and c^S are the mean concentrations in the mucosal and serosal solutions, respectively.

The flows of sodium, potassium, and chloride were calculated from the volume and concentration changes of the serosal solution. The error in the measurement of ion flow is approximately 9%. Water flow was equated to the volume change of the serosal solution. The error in the measurement of initial and final volumes is respectively 1.65 and 0.3%.

The change in the total quantity of any species,, in the serosal solution will be a true measure of its flow, J_i, providing that changes in the tissue content of i are negligible or that they occur between tissue and mucosal solution only. To minimize changes in tissue content, the ileum, after mounting on the apparatus, was allowed to equilibrate for 20 min with the bathing solutions before measurements of ion and water transport were started. In all experiments reported here, the changes in tissue content of sodium and chloride never exceeded 10 μEq during the 60 min measurement period, were sometimes positive (tissue gain), and sometimes negative and, on the average, were about 10% of the observed flows. The maximum change amounted to 22 and 20% of the total tissue content of sodium and chloride, respectively. In the experiments described in Table I, the tissue always lost potassium. The tissue gained an average of 0.02 ml of water during the measurement period in all experiments.

In the experiments in which the ileum was subjected to an applied current (Figs. 1 to 3), the solution bathing each surface of the intestine had the following composition, NaCl 137 mM, KCl 4.8 mM, Na_2HPO_4 2.5 mM, $MgSO_4$ 1.2 mM, $CaCl_2$ 1.2 mM and KH_2PO_4 0.6 mM, glucose 10 mM, and reduced glutathione 1 mM. The pH was adjusted to 7.4 with sodium hydroxide. In experiments described in Table I, sodium and potassium concentrations in the bathing solutions were adjusted to any desired value by replacing sodium chloride with an equivalent quantity of potassium chloride. In the experiments described in Table III, the osmolarity of the bathing solutions was adjusted by changing the concentration of sodium chloride.

The ileal segments were examined histologically at the end of the experiment. The villi were distended, a phenomenon associated with water absorption across the isolated preparation (14). The epithelial cell layer was intact except for the sheeding of the cells at the tips of some of the villi, a normal process in the intact rat (10).

RESULTS

All the experimental observations are presented in Section I grouped according to the nature of the thermodynamic driving force. In Section II, the data are used to test the literature friction model presented in the Introduction and in Section III modifications of the model are considered.

Section I

A. FLOWS UNDER ELECTRICAL DRIVING FORCES The experiments described in this section were designed to study ion and water flows under the simplest possible conditions: the mucosal and serosal solutions had the same composition (mainly NaCl, see Methods); the applied electrical field was the only external driving force; and only three flows need be measured (those of sodium, chloride, and water) since it had been shown previously (13) that the other solutes present in the bathing solutions were not transported in significant quantities.

The voltage and the flows of sodium, chloride, and water across the ileum are presented in Figs. 1, 2, and 3 plotted against the applied current (-8 to $+10$ ma). The flows are defined as positive when in the mucosal to serosal direction and the voltage when the serosol side is positive with respect to the mucosal side. To avoid confusion in comparing the sodium and chloride flows given in Figs. 1 and 2, it should be noted that the ion flows are expressed as positive current flows in milliamperes. Thus when sodium and chloride are each moving in the same direction, the flow of one will be opposite in sign with respect to the other.

The relation between the applied current $I(ma)$ and the voltage V (mv) was linear (Fig. 1) and the quantitative relationship, as determined by linear regression analysis, was

$$V = 4.2I + 7.7 \tag{25}$$

It should be noted that V was corrected for the resistance of the solution as described previously (13). Thus the dc resistance of this preparation of ileum was 4.2 ohms and the open-circuit potential was 7.7 mv. With an estimated

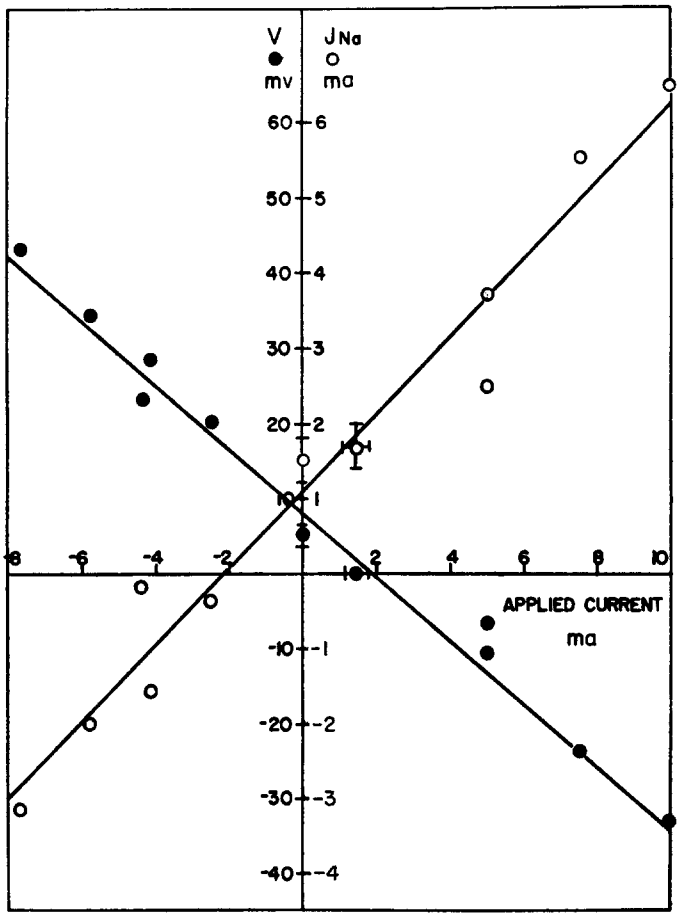

FIGURE 1. The voltage (V) and the sodium flow (J_{Na}) across the ileum are plotted against the applied current. J_{Na} is positive when in the mucosal to serosal direction and V when the serosal side is positive vs. the mucosal side. Each point is one observation. The line was drawn by least squares regression analysis. The ileum was bathed by identical solutions consisting predominantly of NaCl.

surface area of 36 cm² (13), the tissue has a specific transverse resistance (specific resistance times thickness in cm) of 151 ohms cm².

The sodium flow, I_{Na}, was proportional to the applied current. The linear regression line was

$$I_{Na} = 0.51I + 1.1 \tag{26}$$

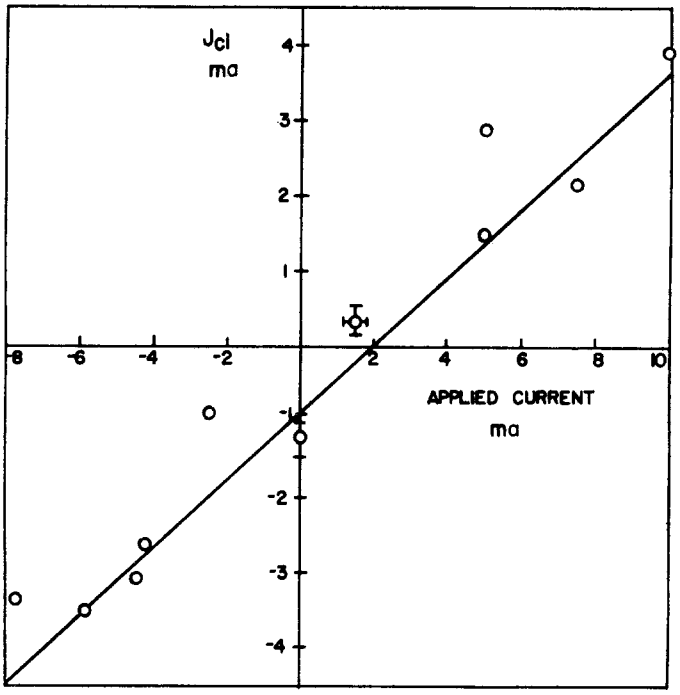

FIGURE 2. The chloride flow, (J_{Cl}), is plotted against the applied current and is expressed in milliamperes of positive current and is positive when in the serosal to mucosal direction. Each point is one observation. The line was drawn by least squares regression analysis. The data are from the same experiments as described in Fig. 1.

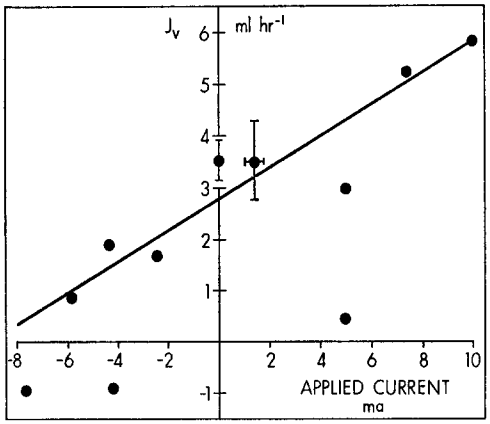

FIGURE 3. The volume flow (J_v) is plotted against the applied current and is positive when in the mucosal to serosal direction. Each point is one observation except the point on the ordinate (six observations) and that at 1.83 ma (five observations). The vertical and horizontal lines drawn from these points are equal to the standard across. The line was drawn by least squares regression analysis. The data are from the same experiments as described in Figs. 1 and 2.

The current required to give zero voltage across the ileum (the shortcircuit current) according to equation (25) was 1.83 ma. The sodium flow at this value of the applied current was 2.07 ma (SE 0.24) according to equation (26). The two numbers do not differ significantly ($P = 0.05$).

The chloride regression line calculated from the data of Fig. 2 is

$$I_{Cl} = 0.44I - 0.96 \tag{27}$$

Chloride flow under short-circuit conditions according to equation (27) was 0.2 ma (SE 0.4) which is insignificantly different from zero.

Comparison of equations (26) and (27) indicates first that sodium and chloride flows account for 95% of the current passing across the ileum, which is to be expected since these ions, together, account for 98% of the ionic strength of the bathing solution, and second, that the transference numbers of sodium and chloride are respectively 0.51 and 0.44. The corresponding numbers in free solution are 0.3 and 0.7. However, these transference numbers cannot be exactly compared to the free solution values since they are uncorrected for bulk flow of solution across the tissue. However, the fact that they are nearly equal does indicate that the fixed charge density in that transport pathway cannot be very high.

Water flow (measured as volume flow, J_s in ml hr^{-1}) was linearly related to the applied current (Fig. 3) according to the regression line

$$J_s = 0.03I + 0.27 \tag{28}$$

In effect this equation describes electroosmotic flow of water across the ileum, suggesting the presence of aqueous pores lined with fixed charge groupings. The direction of the change in J_s in response to a change in I is as if the fixed charge groups were negative in sign.

One point, at an applied current of +5 ma in Fig. 3 was very much off the line. The flows of sodium and chloride were also abnormally low in this experiment (Figs. 1 and 2). The reason for this deviation will be discussed below in relation to Fig. 4.

B. FLOWS UNDER CHEMICAL POTENTIAL GRADIENTS Large flows of sodium and potassium were observed in the presence of concentration (chemical potential) gradients when the electrical gradient was small and virtually constant (Table I). Potassium always moved down the net electrochemical gradient. In one experiment (No. 4) sodium moved against the gradient in confirmation of previous observations (4).

Water transport was small, usually less than 0.15 ml hr^{-1}. This is probably due to the fact that sodium and potassium were moving in opposite directions across the ileum so that the net movement of total solutes was small. The

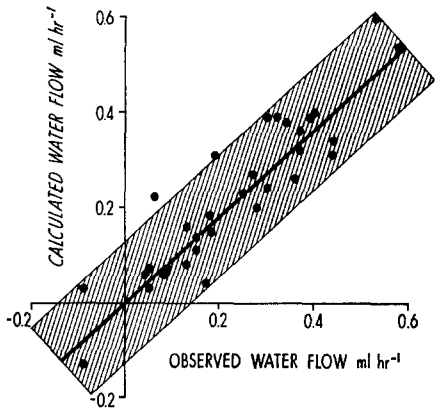

FIGURE 4. The observed water flow, J_v^{obs}, is plotted as a function of ion flow according to equation (24) of the literature friction model. Values of f'_{iw} are taken from Table III. The line was drawn by least squares regression analysis, and the shaded area represents the 95% confidence limits. The data are from the experiments described in Figs. 1 to 3 and Table 1.

TABLE II

SALT AND WATER FLOWS IN THE PRESENCE OF OSMOTIC PRESSURE GRADIENTS

No.	c_s	J_v	c_s^M	c_s^S	J_s	volts
	milliosmols/liter	*ml/hr*	*milliosmols/liter*	*milliosmols/liter*	*milliosmols/hr*	*mv*
1	+173	−0.02	512	339	159	4.4
2	+159	−0.02	502	347	229	2.1
3	+99	+0.09	295	196	122	5.4
4	+93	+0.04	293	200	111	4.8
5	+71	−0.18	395	324	106	6.1
6	−59	+0.46	292	351	12	5.6
7	−61	+0.39	295	356	14	5.0
8	−63	+0.57	295	358	61	7.0
9	−90	+1.21	292	382	146	8.7
10	−100	+0.40	292	392	−45	5.8
11	−158	+0.54	290	448	−180	4.0
12	−161	+0.48	287	444	−214	5.5

The salt flow J_s is the sum of J_{Na} and J_{Cl} and the salt concentration c_s is the sum of $c_{Na} + c_{Cl}$. c_s is the mean concentration gradient of salt, positive when the mucosal solution is at the higher concentration. Each horizontal row is from one experiment. The mean tissue dry weight was 220 mg.

greatest movement of water, 0.37 ml hr^{-1} was seen in experiment 4 where sodium and potassium were moving in the same direction.

In the experiments described in Table II, salt and water flows were measured in the presence of activity gradients of water created by placing different concentrations of sodium chloride on each side of the tissue. There are several notable qualitative aspects of this data. First, in experiments 8 and 9 a net flow of salt took place against the chemical potential gradient; second, water was transported against its activity gradient in experiments 2 and 3 in confirmation of previous reports by Parsons and Wingate (15); and third, the

voltage across the tissue was virtually constant despite large changes in the concentrations of salt. It would appear that NaCl does not produce significant diffusion potentials.

Section II

ION FLOWS

The data of section I may now be used to test the friction model. Specifically, equation (26) should describe ion flows reported in Section I *A* and Table I. When the left-hand side, $\Delta\tilde{\mu}_i$, is plotted against the variable term on the right-hand side, $(J_i^{obs}/\bar{c}_i - J_v^{obs})$, a straight line should be obtained having a gradient of f_{iw} $(\Delta x^P/A^P)$ and an intercept equal to $f_{iw}(\Delta x^P/A^P)(J_i^A/\bar{c}_i - J_v^A)$.

TABLE III
ION-WATER FRICTION COEFFICIENTS

Type of experiment	Ion	f'_{iw}	F'_{iw}
		mv hr ml^{-1}	*mv*
Electrical gradient	Na+	40.5±0.9	−9.5±0.9
	Cl−	28.7±1.9	+9.9±1.0
Chemical gradient	Na+	36.3±2.8	−7.8±1.9
	K−	22.2±0.3	+6.5±0.7

The values of the ion-water coefficient, f'_{iw}, and the residual constant, F'_{iw}, were calculated by linear regression analysis of the data on ion and water flows and the applied electrochemical gradients according to equation (29). The data are from experiments described in Section IA and Table I. The numbers following the ± sign are standard errors.

In fact linear relationships were found for the three ions studied, viz. sodium flowing under either electrical or chemical potential gradients, chloride under electrical potential gradients, and potassium under chemical potential gradients. Linear regression analysis of $(J_i^{obs}/\bar{c}_i - J_v^{obs})$ on the independent variable $\Delta\tilde{\mu}_i$ gave the following general expression

$$\Delta\tilde{\mu}_i = f'_{iw}(J_i^{obs}/\bar{c}_i - J_v^{obs}) + F'_{iw} \tag{29}$$

where f'_{iw} is equivalent to $f_{iw}(\Delta x^P/A^P)$ and F'_{iw} to $f'_{iw}(J_i^A/\bar{c}_i - J_v^A)$. Values for the linear regression gradient f'_{iw} and the intercept F'_{iw} are given in Table III. $\Delta\tilde{\mu}_i$ was calculated according to equation (16) and $\bar{c}_i$ according to equation (14). The method of calculating $\bar{c}_i$ was important only in the case of potassium where the use of equation (14) gave the required linear relation, whereas, for example, $\bar{c}_K$, calculated as the arithmetic mean, gave a nonlinear plot.

Inspection of the values of f'_{iw} given in Table III suggests the following conclusions: (1) The small variability in f'_{iw} and F'_{iw} for the flows of the three

ions studied indicates that equation (22) gives an excellent description of the data. For example, the mean value of f'_{iw} calculated from sodium flows under both electrical and chemical potential gradients was 38.3 mv ml^{-1} hr with a standard error of only ±2.7. The sodium flow ranged from −119 to +240 μEq hr^{-1}, its concentration in the bathing solutions from 18 to 145 mEq l^{-1}, and the driving forces from −46 to +36 mv with electrical gradients and from −29 to +40 mv with chemical potential gradients. (2) The values of f'_{iw} and F'_{iw} calculated from sodium flows under chemical potential gradients are identical to the values calculated from flows under electrical gradients. (3) The values of f'_{iw} are in the inverse ratio of the relative ionic mobilities in free solution. Taking the values of the ionic mobilities of sodium, potassium, and chloride as 5.2, 7.6, and 7.9 cm sec^{-1}, respectively, the corresponding products with f'_{iw} are practically constant, viz. 2.0×10^{-2}, 1.8×10^{-2}, and 2.3×10^{-2}. (4) The numerical values of F_{iw} for the flows of sodium and chloride under electrical driving forces are equal in magnitude but opposite in sign. But it has already been demonstrated in Section I that chloride flow under short-circuit conditions is zero. Consequently, $J^A_{Cl} = 0$. Thus if $F'_{Naw} = -F'_{Clw}$, it follows that

$$f_{Cl_w} J^A_v = f_{Na_w}(J^A_{Na}/\bar{c}_{Na} - J^A_v)$$

or

$$J^A_v = f_{Na_w} J^A_{Na}/(f_{Na_w}\bar{c}_{Na} + f_{Cl_w}\bar{c}_{Cl}) \tag{30}$$

Thus the analysis of the experimental observations according to the literature friction model, indicates that there is a special relationship between sodium and water flows through the active channel as given by equation (30).

Significant amounts of potassium were lost from the tissue in the experiment described in Table I. The value of F'_{K_w} of 6.5 (Table III) assumed that potassium was lost equally from both surfaces of ileum. But if all the loss took place across the mucosal surface, the value of F'_{K_w} would be reduced to 1.8 mv. Accordingly little significance can be attached to F'_{K_w}. Assumptions concerning the direction of potassium loss from the tissue have no effect on the value of f'_{K_w}.

The data of section I were also analyzed by more general friction models than by the one presented here. For example, equation (31) below takes into account the possibility of friction between the ion and the matrix of the passive channel, expressed by the coefficient f^P_{im} and may be derived in the same way as equation (22).

$$\begin{aligned}\Delta\tilde{\mu}_i = {} & f_{iw}(\Delta x^P/A^P)(J^{obs}_i/\bar{c}_i - J^{obs}_v) + f^P_{iw}(\Delta x^P/A^P)J^{obs}_i \\ & - f_{iw}(\Delta x^P/A^P)(J^A_i/\bar{c}_i - J^A_v) - f^P_{im}(\Delta x^P/A^P)J^A_i\end{aligned} \tag{31}$$

Multilinear regression analysis revealed that the mean value of f'_{im} (equal to $(f^P_{im})(\Delta x^P/A^P)$) was not significantly different from zero, ($P = 0.05$), for the flows of sodium and potassium and gave a negative value for chloride.

WATER FLOW

The data on water flow given in Fig. 3 and Table I are from experiments in which hydrostatic and osmotic pressure gradients were absent so that equation (24) is applicable. A graph of J_v^{obs} against $\sum f_{iw}J_i^{obs}/\sum f_{iw}\bar{c}_i$ should give a straight line having a gradient of unity and an intercept equal to $J_v^A - \sum f_{iw}J_i^A/\sum_{iw}\bar{c}_i$. The values of f'_{iw} given in Table III may be used in place of f_{iw} in equation (24) since the terms $(\Delta x^P/A^P)$ contained in f'_{iw} will cancel. The plot is presented in Fig. 4. The slope of the regression line is 0.95 (insignificantly different from unity). The line, however, passes through the origin indicating that the term $J_v^A - \sum f_{iw}J_i^A/\sum f_{iw}\bar{c}_i = 0$. Clearly this equation is a more general expression of equation (32). Consequently, two phenomena have now been observed, each resulting from the same, as yet unknown, property of the active transport channel.

Water flow in the presence of osmotic pressure gradients is given by equation (23). This equation is most conveniently applied to the data of Table II by introducing the following definitions; when the tissue is bathed by salt (sodium chloride) solutions differing only in concentration, we may write $J_i^{obs} = J_{Na}^{obs} = \frac{1}{2} J_s^{obs}$ and $\bar{c}_i = \bar{c}_{Na} = \bar{c}_{Cl} = \frac{1}{2}\bar{c}_s$ where the flow of salt J_s is expressed in milliosmols hr^{-1} and the salt concentration, $\bar{c}_s$ in milliosmoles l^{-1} since J_v is given in ml hr^{-1}. Defining f_{sw} as the sum of $f_{Na_w} + f_{Cl_w}$, and knowing that $J_{Cl}^A = 0$ (Section I), equation (23) becomes, on introducing the above relations,

$$-\Delta\pi s/\mathfrak{F} = (\Delta x^P/A^P)f_{sw}(J_v^{obs}\bar{c}_s/2 - J_s^{obs}/2) - (\Delta x^P/A^P)f_{sw}J_v^A\bar{c}_s/2 + (\Delta x^P/A^P)f_{Na_w}J_{Na}^A \tag{32}$$

Since $\Delta\pi_s = RT\Delta c_s$, equation (32) becomes on rearrangement

$$(2\,RT/\mathfrak{F})\Delta c_s/\bar{c}_s = 2\Delta\mu_s = (\Delta x^P/A^P)f_{sw}(J_s^{obs}/\bar{c}_s - J_v^{obs}) - (\Delta x^P/A^P)f_{Na_w}J_{Na}^A/\bar{c}_{Na} - (\Delta x^P/A^P)f_{sw}J_v^A \tag{33}$$

F is introduced in equation (32) so that $\Delta\mu_s$ (equation 31) will be in millivolts and f_{sw} and f_{Na_w} will have the same units as in the previous equations. The term on the left-hand side is measurable and expresses the chemical potential gradient of sodium chloride in millivolts. When this term is plotted against the variable term on the right-hand side $(J_s^{obs}/\bar{c}_s - J_v^{obs})$, a straight line should be obtained of gradient $f_{sw}\Delta x^P/A^P$, and having an intercept of $f_{sw}(\Delta x^P/A^P)J_v^A - f_{Na_w}(\Delta x^P/A^P)J_{Na}^A/\bar{c}_{Na}$. Fig. 5 indicates that this plot is in

fact linear, over a wide range of chemical potential gradients (+10 to −29 mv). The points which deviate (open circles, Fig. 5) are from experiments in which the osmolarity of the bathing solutions showed the greatest deviation from isotonicity (less than 240 or greater than 420 milliosmols). These points were not included in the calculation of the regression line. Data from similar experiments on rat ileum, reported by Parsons and Wingate (15) gave a straight line which passes through the origin. Their data deviate from the linear relationship at the same value of $\Delta\mu_s$ and probably for the same reason.

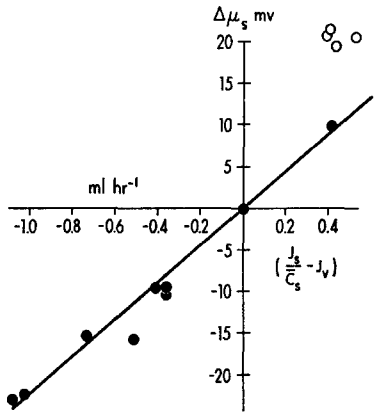

FIGURE 5. The flow of salt (J_s) and the volume flow (J_v) are plotted against the chemical potential gradient of salt ($\Delta\mu_s$) according to equation (33). J_s is milliequivalents per hour and $\bar{c}_s$, the mean of the salt concentrations in the mucosal and serosal solutions, is in milliosmols per milliliter so that $J_s/\bar{c}_s$ is in milliliters per hour. The open circles are from experiments in which the osmolarity of the bathing solutions was less than 240 or greater than 420 milliosmols. The linear regression line was calculated from the data represented by solid circles. The data were taken from Table II except for the point at the origin which is a mean value of all the data from Fig. 4 where $\Delta\mu_s$ was zero.

Two important differences between the observed regression line of Fig. 5 and the line predicted by equation (33) should be noted. First, the gradient of the regression line is 22 whereas, according to equation (33) the gradient should be 67 ($(\Delta x^P/A^P)f_{sw} = f'_{sw} = f'_{\mathrm{Na}_w} + f'_{\mathrm{Cl}_w}$ using values of f'_{Na_w} and f'_{Cl_w} given in Table III); second, the regression line passes through the origin. Both differences from equation (33) indicate that the term assumed to be constant in (33) is in fact proportional to $\Delta\mu_s$. Thus, if α is the proportionality constant, we may write

$$\Delta\mu_s\,\alpha = (\Delta x^P/A^P)(f_{\mathrm{Na}_w} J^A_{\mathrm{Na}}/\bar{c}_{\mathrm{Na}} - f_{sw} J^A_v) \tag{34}$$

Introducing equation (34) into (33) we obtain

$$\Delta\mu_s(1 - \alpha) = (\Delta x^P/A^P)f_{sw}(J^{\mathrm{obs}}_s/\bar{c}_s - J^{\mathrm{obs}}_v)$$

giving an expression entirely compatible with the observed regression line. Of more importance, it should be noted that, when $\Delta\mu_s = 0$, equation (34) reduces to equation (30). Thus in this case also, the difference between the observed line and the predicted line appears to result from a special relationship between the sodium and water flows through the active channel.

Section III

At this point, it may be helpful to recapitulate the main conclusions from Sections I and II. The first section indicated that sodium and water are transported under short-circuit conditions and chloride is not, and that, when large voltage differences are placed across the tissue, some water is transported by electroosmosis indicating the presence of aqueous pores lined with negative fixed charge groupings. Section II indicated that the literature friction model gives an adequate description of ion flows under electrical and chemical potential gradients, and correctly predicted the gradients of the regression line in Fig. 4 describing water flow in the absence of osmotic pressure gradients. However, three inadequacies of the model have been noted: the failure to predict that $F_{Cl_w} + F_{Na_w} = 0$ (Table III), that the regression lines of Figs. 4 and 5 pass through the origin, and finally the failure to predict the correct gradient of the regression line in Fig. 5. As indicated in Section II these inadequacies may be due to a single property of the active transport channel expressed by equations (30) and (34). These equations are formally similar to the equation (18) describing water flow through the passive channel. They differ from equation (18) in that ion flow is restricted to the active flow of sodium J^A_{Na}. In other words, all three inadequacies are an expression of the fact that the chief resistance to water flow through the active channel is friction with the ions in the channel. It will be recalled that the literature friction model included no statement about the interactions between ion and water flows through the active channel but assumed only that each flow was constant.

Moreover, in designing a specific model for the active transport channel, we must also take into account the fact that the isolated intestine is able to transport water against its activity gradient as observed here (Table II) and previously by Parsons and Wingate (15). Electroosmosis through the passive channel cannot account for this because the voltage gradient across the tissue is in the wrong direction. The model of the active transport channel proposed below attempts to take into account all the above considerations in the simplest possible way.

A. FRICTION MODEL OF ACTIVE TRANSPORT

The active channel is assumed to consist of two membranes in a series. The first membrane (I) is, for all practical purposes, semipermeable, but allows active transport of sodium across it at a constant rate, J^A_{Na}, in the mucosal to

serosal direction, and the second membrane (II) is similar to the passive channel and is situated on the serosal side of the membrane (I). The chemical potential difference of water across the ileum, Δ_{μ_w}, will be related to the chemical potential differences, $\Delta\mu_w^{\mathrm{I}}$ and $\Delta\mu_w^{\mathrm{II}}$, across membranes I and II by the equation

$$\Delta\mu_w = \Delta\mu_w^{\mathrm{I}} + \Delta\mu_w^{\mathrm{II}} \tag{35}$$

Assuming that the water experienced friction with the matrix of membrane (I) but not with the actively transported sodium, the integrated flow equation for membrane (I) is (see Kedem and Katchalsky (6), equations (1–15) where $f_{sw} = 0$),

$$\Delta\mu_w^{\mathrm{I}} = f_{wm}^{\mathrm{I}}(\Delta x^{\mathrm{I}}/A^{\mathrm{I}})J_v^A \tag{36}$$

where Δx^{I} and A^{I} are the length and area of membrane (I), respectively, and J_v^A, the volume flow through the active channel. J_v^A will be the volume flow through both membranes since the latter are in series.

Since the properties of membrane II are assumed to be identical to those of the passive channel, equation (9) is applicable. On dividing both sides of the equation by C_w and replacing C_w by c_w according to equation (10) and taking the distribution coefficient k_w to be unity, equation (9) becomes an integration across membrane II

$$\Delta\mu_w^{\mathrm{II}} = (\Delta x^{\mathrm{II}}/A^{\mathrm{II}}\bar{c}_w)f_{iw}(J_v^A\bar{c}_i^{\mathrm{II}} - J_i^A) \tag{37}$$

where $\bar{c}_w$ is defined as in equation (12) and $\bar{c}_i$ as in equation (19). Applying equation (36) to the experiments (Table II) in which the intestine is bathed by sodium chloride solutions and introducing the definitions given for equation (32), we obtain

$$\Delta\mu_w^{\mathrm{II}} = (\Delta x^{\mathrm{II}}/A^{\mathrm{II}}\bar{c}_w)(f_{sw}J_v^A\bar{c}_s^{\mathrm{II}}/2 - f_{\mathrm{Na}_w}J_{\mathrm{Na}}^A) \tag{38}$$

Adding equations (36) and (37) according to equation (35) we obtain

$$\begin{aligned}\Delta\mu_w &= \Delta\mu_w^{\mathrm{I}} + \Delta\mu_w^{\mathrm{II}} \\ &= (\Delta x^{\mathrm{I}}/A^{\mathrm{I}})f_{wm}^{\mathrm{I}}J_v^A + (\Delta x^{\mathrm{II}}/A^{\mathrm{II}}\bar{c}_w^{\mathrm{II}})(f_{sw}J_v^A\bar{c}_w^{\mathrm{II}}/2 - f_{\mathrm{Na}_w}J_{\mathrm{Na}}^A) \\ &= J_v^A\{(\Delta x^{\mathrm{I}}/A^{\mathrm{I}})f_{wm}^{\mathrm{I}} + (\Delta x^{\mathrm{II}}/A^{\mathrm{II}}\bar{c}_w^{\mathrm{II}})f_{sw}\bar{c}_s^{\mathrm{II}}/2\} + (\Delta x^{\mathrm{II}}/A^{\mathrm{II}}\bar{c}_w^{\mathrm{II}})f_{\mathrm{Na}_w}J_{\mathrm{Na}}^A\end{aligned} \tag{39}$$

Assuming that $(\Delta x^{\mathrm{I}}/A^{\mathrm{I}})f_{wm}^{\mathrm{I}}$ is small compared to $(\Delta x^{\mathrm{II}}/A^{\mathrm{II}}\bar{c}_w^{\mathrm{II}})f_{sw}\bar{c}_s^{\mathrm{II}}$, equation (39) becomes

$$\Delta\mu_w = -(\Delta x^{\mathrm{II}}/A^{\mathrm{II}}\bar{c}_w^{\mathrm{II}})(f_{\mathrm{Na}_w}J_{\mathrm{Na}}^A - f_{sw}J_v^A\bar{c}_s^{\mathrm{II}}/2) \tag{40}$$

This assumption states that membrane II presents the predominant resistance to water flow through the active channel and that membrane I affects water movement indirectly by acting as a source of active transport, J^A_{Na} and restricts all other ion flows through the channel. Since f^I_{wm} cannot be expected to be insignificant, the relative dimensions of the two membranes must counteract this, viz.

$$\Delta x^I/A^I \ll x^{II}/A^{II}.$$

The explicit thermodynamic expressions for $\Delta\mu_s$ and $\Delta\mu_w$ are

$$\Delta\mu_s = \bar{V}_s\Delta p + \Delta\pi_s/\bar{c}_s \tag{41}$$

$$\Delta\mu_w = \bar{V}_w(\Delta p - \Delta\pi_s) \tag{42}$$

where $\Delta\pi_s$ is the osmotic pressure difference and $\bar{c}_s$ is defined as

$$\bar{c}_s = \Delta\pi_s/RT\Delta \ln \bar{c}_s \tag{43}$$

Under the experimental conditions used in this work, the hydrostatic pressure difference Δp is zero. Thus the relationship between $\Delta\mu_s$ and $\Delta\mu_w$ is

$$\Delta\mu_w = -\bar{V}_w\Delta\mu_s\bar{c}_s \tag{44}$$

Replacing $\Delta\mu_w$ by $\Delta\mu_s$ in equation (40), we obtain

$$\Delta\mu_s = (\Delta x^{II}/A^{II}\bar{V}_w\bar{c}^{II}_w)(f_{Na_w}J^A_{Na}/\bar{c}_s - f_{sw}J^A_v\bar{c}^{II}_s/\bar{c}^2_s) \tag{45}$$

where $\bar{V}_w$ and $\bar{c}_s$ are the partial molal volume of water and the mean salt concentration, respectively, in the bathing solutions. Since membrane II is in equilibrium at its serosal end with the serosal solution and is separated from the mucosal solution by a membrane readily permeable to water, it may be assumed that $\bar{V}_w\bar{c}^{II}_w = \bar{V}_w\bar{c}_w = \bar{c}^{II}_s/C_s = 2C^{II}_{Na}/\bar{c}_s = 1$. Introducing this approximation, equation (45) becomes

$$2\Delta\mu_s = (\Delta x^{II}/A^{II})(f_{Na_w}J^A_{Na}/\bar{c}_{Na} - f_{sw}J^A_v) \tag{46}$$

B. THE MODIFIED FRICTION MODEL

The complete model consists of two sets of parallel pathways. The passive channel is defined in the literature model and the active transport channel is defined in Section 3 A above (equations 35 to 46). The observed flows across the ileum will be the algebraic sum of the flows through each set of channels as defined by equations (20) and (21). The modified friction model will be applied to the data of this report which the literature friction model failed to describe adequately, and also to observations reported in the literature.

1. *Volume Flow in the Absence of Osmotic Pressure Gradients* The fact that the regression line of Fig. 4 passes through the origin may be explained as follows. In the absence of osmotic pressure gradients, $\Delta\mu_w = \Delta\pi_s = 0$ and the equations of flow through the passive and active channels (18 and 46) become, on rearrangement

$$J_v^P = \sum_i f_{iw} J_i^P / \sum_i f_{iw} \bar{c}_i \tag{47}$$

and

$$J_v^A = f_{Na_w} J_{Na}^A / \sum f_{iw} \bar{c}_i \tag{48}$$

TABLE IV

CALCULATED AND OBSERVED WATER FLOWS UNDER SHORT-CIRCUIT CONDITIONS

No. of experiments*	Ion flow		Water flow	
	J_{Na}	J_{Cl}	Calculated‡	Observed
	μEq/hr		*ml/hr*	
7	66	−2	0.26	0.25
5	67	14	0.30	0.35
4	125	−34	0.38	0.40

* Each group of experiments was carried out with a different concentration of a thiol compound which was added to the solutions to protect the tissue from silver ions released from the electrode. The experiments were reported in detail previously (13).

‡ Calculated according to equation (48).

Adding equations (47) and (48) according to equation (21) we obtain

$$\begin{aligned} J_v^{obs} = J_v^P + J_v^A &= \sum_i f_{iw}(J_i^P + J_i^A / \sum_i f_{iw} \bar{c}_i \\ &= \sum_i f_{iw} J_i^{obs} / \sum_i f_{iw} \bar{c}_i \end{aligned} \tag{49}$$

Equation (49) is exactly the relationship given by the regression line in Fig. 4. In the experiments in which the intestine is bathed by identical solutions of NaCl under open-circuit conditions equation (49) becomes

$$J_{vi}^{obs} = J_s / \bar{c}_s \tag{50}$$

which states that the osmolarity of the transported solution is identical to that of the bathing media. Experiments both in vitro (4) and in vivo (3) indicate that the transported solution possesses an osmolarity very close to that of the ambient solutions. Small deviations from equation (50) are to be expected due to the release of lactic acid and small movements of other ions (13).

When the ileum is short-circuited, $\Delta\bar{\mu}_i$ and $\Delta\mu_w$ are both zero, so no movement of ions or water is possible through the passive channel. Consequently, the observed ion and water flows under short-circuit conditions should be equal to the flows through the active channel and should be described by equation (48). The coefficients f'_{iw} given in Table III may be used in equation (48) since the $(\Delta x/A)$ terms cancel. The water flows calculated by equation (48) from the observed ion flows in the short-circuit experiments of Clarkson and Toole (13) agree well with the observed values of J_v (Table IV).

2. *Volume Flow in the Presence of Osmotic Pressure Gradients* The flows of salt and water reported in Table II took place in the presence of osmotic pressure gradients when the intestine was bathed by sodium chloride solutions under open-circuit conditions. Flows through the active channel are given by equation (46). The flow equations for sodium and chloride for the passive channel are given by equation (15) as

$$\Delta\mu_{Na} - \Delta\psi = (\Delta x^P/A^P)f_{Na_w}(J^P_{Na}/\bar{c}_{Na} - J^P_v) \tag{51}$$

$$\Delta\mu_{Cl} + \Delta\psi = (\Delta x^P/A^P)f_{Cl_w}(J^P_{Cl}/\bar{c}_{Cl} - J^P_v) \tag{52}$$

Adding equations (51) and (52) and introducing the definitions given for equation (32) we obtain

$$2\Delta\mu_s = (\Delta x^P/A^P)(f_{Na_w}J^P_{Na}/\bar{c}_{Na} + f_{Cl_w}J^P_{Cl}/\bar{c}_{Cl}) - (\Delta x^P/A^P)f_{sw}J^P_v \tag{53}$$

Dividing equations (46) and (53) by $(\Delta x^{II}/A^{II})$ and $(\Delta x^P/A^P)$, respectively and adding, we obtain

$$2\Delta\mu_s(A^{II}/\Delta x^{II} + A^P/\Delta x^P) = f_{Na_w}(J^P_{Na} + J^A_{Na})\bar{c}_{Na} + f_{Cl_w}J^P_{Cl}/\bar{c}_{Cl} - f_{sw}(J^P_v + J^A_v) \tag{54}$$

Introducing equations (20) and (21), equation (54) becomes under open-circuit conditions

$$2\Delta\mu_s(A^{II}/\Delta x^{II} + A^P/\Delta x^P) = f_{sw}(J^{obs}_s/\bar{c}_s - J^{obs}_v) \tag{55}$$

Equation (55) describes the flow of salt and water in the presence of osmotic pressure gradients and indicates that the regression line of Fig. 5 should have a gradient of $f_{sw}/(A^{II}/\Delta x^{II} + A^P/\Delta x^P)$ which may be rewritten as $f'_{sw}/\{1 + (\Delta x^P/A^P)/(\Delta x^{II}/A^{II})\}$. Taking the value of f'_{sw} as 67 (values of f'_{Na_w} and f'_{Cl_w} were taken from Table III) the regression gradient of Fig. 5 indicates that $(\Delta x^P/A^P)/(\Delta x^{II}/A^{II}) = 2$. Parenthetically it should be recalled that the literature friction model predicted the gradient to be f'_{sw} which was incompatible with the observed value of 22.

The modified friction model is clearly compatible with the data of Fig. 5, correctly predicting a straight line passing through the origin. Unfortunately no data are available to check independently the value of the ratio $(\Delta x^P/A^P)/(\Delta x^{II}/A^{II})$ as calculated above. The value of this ratio may possibly be checked by microelectrode measurements since this ratio allows a prediction of the electrical potential profile across the active transfer channel from mucosal to serosal sides. The ion-water friction coefficient f_{iw}^{II} of membrane II of the active channel is defined as

$$f_{iw}^{II} = f_{iw}(\Delta x^{II}/A^{II}) = f'_{iw}(\Delta x^{II}/A^{II})/(\Delta x^P/A^P) = \tfrac{1}{2}f'_{iw} \quad (56)$$

Equations 51 and 52 are applicable to membrane II since its properties are identical to those of the passive channel. Considering the case in which the intestine is bathed by identical solutions of sodium chloride, the equations may be solved for $\Delta\mu_i$ and $\Delta\psi^{II}$ since the values for ion and water flows are known from Section I (i.e. the flows under short-circuit conditions) and the ion-water frictions are given in Table III. The calculation indicates that the chemical potential gradient across membrane (II) is zero and the electrical gradient is 4 mv, the serosal side being negative. Consequently, with an open-circuit voltage across the ileum of 7.7 mv, serosal side positive (see equation (25) Section I), the voltage across membrane I must be +12 mv. In other words, there must exist a locus inside the ileum, representing the junction of membranes I and II which is at a higher positive potential than the serosal aspect. This prediction could be checked by direct measurement.

3. *The Significance of the Constants, F'_{iw}, in Table III* Equation (22) describes the observed ion flow under electrochemical potential gradients. Clearly the constant term of equation (29), F'_{iw}, is equal to the last term of equation (22). Since sodium is the only ion actively transported, F'_{iw} is a function of active sodium transport and the values of the ion-water friction coefficients. This is readily demonstrated by considering the case of the intestine bathed by identical solutions of sodium chloride. Under these conditions, equation (22) gives, on substituting for J_v^A from equation (48), the following equation describing the flows of sodium chloride

$$\Delta\tilde{\mu}_{Na} = f_{Na_w}(\Delta x^P/A^P)(J_{Na}^{obs}/\bar{c}_{Na} - J_v^{obs}) - (\Delta x^P/A^P)f_{Na_w}f_{Cl_w}J_{Na}^A/(f_{Na_w} + f_{Cl_w}) \quad (57)$$

and

$$\Delta\tilde{\mu}_{Cl} = f_{Cl_w}(\Delta x^P/A^P)(J_{Cl}^{obs}/\bar{c}_{Cl} - J_v^{obs}) + (\Delta x^P/A^P)f_{Na_w}f_{Cl_w}J_{Na}^A/(f_{Na_w} + f_{Cl_w}) \quad (58)$$

Thus F'_{Na_w} and F'_{Cl_w} as calculated for Table III correspond to the last term in equations (57) and (58), that is $(\Delta x^P/A^P)f_{Na_w}f_{Cl_w}J_{Na}^A/(f_{Na_n} + f_{Cl_w})$. Clearly equations (57) and (58) predict that F'_{Na_w} and F'_{Cl_w} must be equal in magnitude but opposite in sign in agreement with the observed values in Table III.

In the general case in which the intestine is bathed by a variety of monovalent ions, equations (22) and (48) lead to the expression

$$\sum_i F'_{Na_w} \bar{c}_i = 0 \tag{59}$$

4. *The Open-Circuit Voltage in the Presence of an Impermeant Anion* When frog skin or toad bladder is transferred from a solution containing chloride to one containing sulfate as the predominant anion, the open-circuit voltage across the tissue increases substantially. This increase in voltage is due to the increase in the dc resistance of the tissue since chloride is a permeant and sulfate an impermeant anion (16). Like frog skin and toad bladder, the small intestine is freely permeable to chloride and impermeable to sulfate and actively transports sodium. Nevertheless, the open-circuit voltage does not change when sulfate replaces chloride in the ambient media (17).

This perplexing observation may be understood in terms of the modified friction model. When the intestine is bathed by identical solutions of sodium chloride, the open-circuit voltage $(\Delta\psi oc)_{Cl}$ is given by equation (22) as

$$-(\Delta\psi oc)_{Cl} = f'_{Na_w}(J^{obs}_{Na}/\bar{c}_{Na} - J^{obs}_v) - f'_{Na_w}(J^A_{Na}/\bar{c}_{Na} - J^A_v) \tag{60}$$

where $(J^A_{Na})_{Cl}$ is the sodium flow through the active channel. But equation (50) states that $J^{obs}_{Na}/\bar{c}_{Na} = J^{obs}_s/\bar{c}_s = J^{obs}_v$ so that equation (60) becomes

$$-(\Delta\psi oc)_{Cl} = f'_{Na_w}\{(J^A_{Na})_{Cl}/\bar{c}_{Na} - (J^A_v)_{Cl}\} \tag{61}$$

When bathed by sodium sulfate solutions, no net sodium transport is possible since sulfate is impermeant ($J^{obs}_{Na} = 0$). Consequently, the amount of sodium transported through the active channel, $(J^A_{Na})_{SO_4}$, must be balanced by an equal but opposite flow through the passive channel. Since the dependence of water flow on ion flow is the same in both active and passive channels, the water flows through these channels must be equal and opposite. Consequently, J^{obs}_v is zero and the open-circuit voltage in the presence of sulfate, $(\Delta\psi oc)_{SO_4}$, is

$$-(\Delta\psi oc)_{SO_4} = f'_{Na_w}\{(J^A_{Na})_{SO_4}/(\bar{c}_{Na}) - (J^A_v)_{SO_4}\} \tag{62}$$

Equations (61) and (62) indicate that the effect of sulfate on the open-circuit potential will be mediated only in terms of its effect on active transport processes. This effect is probably small since the passive channel is permeable to chloride as well as to sulfate.

5. *The Determination of Ion and Water Frictions by Flux Ratio Measurements* The series membrane model proposed here requires that the first membrane should be practically impermeable to chloride. This postulate is supported by the observations of Schultz, Zalusky, and Gass (18). These workers observed

that the flux ratio of chloride across the short-circuited rabbit ileum was unaffected by changes in volume (water) flow. If the active transport channel is impermeable to chloride, the observed flux ratios must be due entirely to exchange of isotopic chloride through the passive channel whereas volume flow under short-circuit conditions takes place exclusively through the active transport channel. Thus in these experiments, no solvent drag effects are to be expected.

On the other hand, Schultz et al. (18) found that the flux ratio of chloride corresponded to that predicted from the electrochemical gradient when a voltage difference of 25 mv was present across the rabbit ileum, the serosal side being negative. According to the present model this voltage difference should cause bulk flow (electroosmosis) through the passive channel. The question arises whether the magnitude of the water flow is sufficiently great to cause significant deviation of the flux ratio. Equations (25) and (28) indicate that the electroosmotic flow due to an applied voltage of 25 mv is 0.18 ml hr^{-1}. The solvent drag force arising from this flow is $f'_{Cl_w} \times 0.18 =$ 4.8 mv so that the electrochemical gradient corrected for the opposing solvent drag force is 20 mv. The predicted flux ratio for a gradient of 25 mv is 0.39 and for 20 mv is 0.46. The observed flux ratio of 0.40 (SE = 0.3) reported by Schultz et al. (18) does not differ significantly from either of these calculated values.

Several conclusions follow from the above considerations: first, the observations by Schultz et al. (18) confirm that chloride ions do not experience significant friction with water flow through the active channel; second, that the failure to observe solvent drag effect at 24 mv is not inconsistent with friction between ions and water being the predominant frictional interaction in the passive channel; and third, that to observe solvent drag effects on chloride or other ions, high voltage differences will be necessary—of the order of 40 mv or more.

DISCUSSION

The open-circuit voltage across the ileum (7.7 mv), the equality between sodium flow and short-circuit current are in agreement with previous observations on this preparation of rat ileum (13, 19), and also with observation on isolated rabbit ileum (20). The finding that this preparation transports water against its activity gradient (Table III) confirms previous observations by Parsons and Wingate on rat ileum (15) and by Hakim, Lester, and Lifson (21) on dog ileum. The linear relationship between net sodium flow and applied current reported here (Fig. 1) confirms a previous observation (22) on isolated rabbit ileum.

The data in Table V allow comparison of the intestine with other epithelial tissues. The list of tissues included in Table V was chosen on the basis that

all showed the same general mechanism of salt transport; namely that the short-circuit current is carried by the sodium flow. A general pattern is apparent. Tissues, which, in vivo, separate solutions of very different ionic strength and composition (e.g. toad bladder and frog skin), possess a high resistance, a large open-circuit voltage, and a low short-circuit current. Epithelia which transport large volumes of salt solutions and which in vivo are bathed by solutions of similar composition (e.g. kidney proximal tubule and the small intestine) have low resistances and open-circuit voltages but relatively high short-circuit currents.

TABLE V

ELECTRICAL PARAMETERS OF EPITHELIAL TISSUES

Tissue	Species	Short-circuit current	Voltage open-circuit	Resistance	Reference
		$\mu a/cm^2$	*mv*	*ohm* cm^2	
Kidney proximal tubule	Rat	357	18	50	(24, 23)
Ileum*	Rabbit	100	8	58	(20, 18)
Small intestine*	Rat	120–300	8	28–63	(41, 42)
Bladder	*Bufo marinus*	40	20–50	500–1250	(25)
Bladder	Turtle	17–40	50	1750	(26)
Bladder	*Bufo bufo*	14	5–20	357–1428	(25)
Skin	Frog	20	50	4800	(27)

* The short-circuit current and resistances are expressed in relation to the serosal surface area. Values in the report are based on mucosal surface area. Exact comparison is difficult because some intestinal preparations (20, 18, and 42) are roughly planar sheets, whereas others (41 and this report) have a cylindrical shape. The resistance of the 8 cm length of ileum reported here is 4.1 ohms as compared to approximately 2 ohms for the 8 cm length of "middle fifth" segment reported by Barry, Smyth, and Wright (41).

1. *Passive Transport and Extracellular Pathways*

The results strongly support the premise set forth in the derivation of the literature friction model that passive transport of ions takes place through aqueous channels whose radii are large compared to the hydrated ionic radii of sodium, potassium, and chloride. The phenomenological equations indicated that friction between ions and water in the channel was the predominant frictional force, that the ionic concentration in the channel is similar to that in the bathing solutions, and that the values of the ion-water frictional coefficients, f'_{iw} are in the inverse ratio of the corresponding ionic mobilities in free solution.

74% of the cells lining the intestine are lost each day (10). This means that one cell per two thousand will leave the tissue each minute. Microscopic observations *in situ* indicate that, once a cell has exfoliated from the tip of the villus, the gap is closed by neighboring cells "within a very few minutes" (11).

Assuming that the average gap closing time is 1 min, the gaps due to cellular loss would occupy one two-thousandth of the total area of the ileum. The question arises as to whether such a gap, having the dimensions of an epithelial cell, filled with isotonic saline and in numbers occupying at least one two-thousandth the surface area of the ileum, can quantitatively account for the observed electrical resistance, the numerical values of f'_{iw} for sodium, potassium, and chloride, and the rate of electroosmosis.

Calculations given in the Appendix indicate that the electrical resistance and the values of f'_{iw} (Table II) require the aqueous channel to have an area to length ratio ($A/\Delta x$) of approximately 15 cm. Since the average length of the epithelial cell is 20 μ (28), A must be 0.03 cm^2. With an intestinal surface area of 36 cm^2, the fractional area occupied by the channels would be 1/1200.

The electroosmotic flow (0.03 ml hr^{-1} ma^{-1}) indicates a zeta potential of 14 mv and a surface-charged density of 3000 esu cm^{-2} (see Appendix). These figures are compatible with the well known fact that the intestinal cells are covered by a protective layer of mucus. The mucous secretions of the intestine have a chemical composition similar to and share the antigenic properties of, the mucopolysaccharides which determine the electrophoretic and antigenic properties of the surface of the red cell (29). The surface-charged density of the red cell determined electrophoretically is 3700 esu cm^{-2} (30).

Despite the presence of negative fixed-charge groups the composition of the solution in the pore will be practically equal to that of the bathing solutions. Calculations (see Appendix) indicate that a surface-charged density of 3000 esu cm^{-2} will increase the sodium concentration by no more than 0.1%.

The present data do not exclude the possibility that the electroosmotic effects may be due to fixed charges in the basement membrane or include a contribution from such charges. Moreover, for species very much larger than sodium or chloride or for multivalent ions, the basement membrane may constitute the rate-limiting barrier to diffusion. From measurements of mannitol diffusion in vivo across the rat ileum, Curran and Solomon (3) calculated an equivalent pore radius of 36 A. The intestine is practically impermeable to sulfate (17) and poorly permeable to other divalent ions (31). The mucopolysaccharide component of the basement membrane may present a local charged density sufficient to reduce the movement of divalent ions but leave monovalent ions virtually unaffected. Hirsch-Ayalon (32) has described an artificial membrane completely impermeable to sulfate, permeable to sodium and potassium, and very permeable to water.

2. *The Active Transport Channel*

The model of the active transport channel proposed here is generally similar to that previously outlined by Curran (5) and subsequently described in greater mathematical detail by Patlak, Goldstein, and Hoffman (33) to

explain water transport against its activity gradient across intestine, and to the model proposed by Diamond (34) to explain the dependence of water flow on active salt flow across gall bladder. The present studies, in supporting a double membrane model for the active transport channel, indicate that water flow is dependent upon active sodium transport. The results also indicate a property of the active transport system hitherto unsuspected; namely, that the rate-determining resistance to water is the frictional interaction with the ions present in the second membrane. The evidence for this conclusion is given in detail in Sections I and II of the Results and will only be briefly recapitulated here: first, that the regression lines of Figs. 4 and 5 pass through the origin, second that, when the tissue is bathed by identical solutions of sodium chloride, $F'_{Na_w} + F'_{Cl_w} = 0$ (Table III), and third, the observation (17) that the open-circuit voltage across the ileum is not affected by replacing all the permeant anions in the bathing media by an impermeant anion.

Since membrane II of the active channel is the site of the rate-determining resistance to water flow, the present observations cannot give any information on the forces which drive water across membrane I. The double membrane models described in the literature require that the osmolarity of the solution in membrane II should be higher than that of the mucosal solution and so give rise to water flow across membrane I by osmosis. In the derivation of the modified friction model given here, the solution in membrane II was assumed to be isosmolar with the bathing solutions, specifically that $\bar{c}_s^{II}/\bar{c}_s = 1$ (see equations 45 and 46). But in view of the experimental error of approximately 10% in the measurement of net water flows, these results cannot specify the osmolarity in membrane II with an uncertainty of less than 10%. A difference in osmolarity of 10% across membrane I is equivalent to an ideal osmotic pressure of 30 cm of mercury. Probably pressures very much less than this are sufficient to drive water across membrane I. Also the results do not exclude the presence of electroosmotic pressures. Calculations in Section III of the Results indicate a voltage difference of 4 mv across membrane II, serosal side negative. Negative fixed-charged groups in this membrane would give rise to an electroosmotic pressure across membrane I in the right direction. A fixed-charged density sufficient to give a 1% difference in the concentration of cations and anions in membrane II would not be detected by the analytical methods currently available. Parenthetically, it may be noted that the electroosmotic flow through the passive channel was produced by a fixed-charge density giving only a 0.1% difference between the concentrations of sodium and chloride. In summary, the results are compatible with local osmosis as described by Diamond (34) or electroosmosis as it exists in the passive channel or more likely a combination of both, as the forces causing water flow across membrane I.

Any identification of membranes I and II of the active channel with ana-

tomical structures of the intestine must be highly speculative. Curran (35) has suggested that membrane I may represent the serosal facing membrane of the epithelial cell since the latter is probably the site of active sodium transport. He further suggested that membrane II "could be represented by any combination of spaces and diffusion barriers between the cells and at the serosal aspect." The results, given in the report, are compatible with these suggestions. Obviously the type of experiments described here cannot distinguish between the serosal and mucosal membranes of the epithelial cell. Consequently, the mucosal cell layer as a whole may function as membrane I. There is, however, a distinct possibility that only a fraction of the total number of epithelial cells transport sodium at a significant rate. Other cells, perhaps located in the crypts of Lieberkühn, may be inactive. On the other hand, all the cells may be readily permeable to water. Such a situation would be completely compatible with the properties of membrane I as described in the modified friction model which postulates that no interaction takes place in membrane I between water flow and the actively transported sodium.

3. *General Conclusions*

Certain general properties of the intestine arising from the active and passive channels separately or resulting from an interaction between the two channels are worth noting. First, when the intestine is bathed by identical solutions, the osmolarity of the fluid transported through the active channels may be calculated from equation (46), as $(f'_{Naw} + f'_{CLw})/f'_{Naw} = 67/38$ times the osmolarity of the bathing solutions. On the other hand, the osmolarity of the total fluid transported (active plus passive flows) is exactly isosmotic (equation (50)). Thus the effect of the presence of the passive channel is to give isosmotic fluid transport. The possibility that mucosal cells probably vary in their absorptive activities may be related to this point. Cells in the crypts are in an early stage of development and isolated from the solutes in the lumen. Thus the most active cells are probably near the tips of the villi and therefore closest to the passive channel. This should allow efficient "coupling" between the two channels, thereby facilitating osmotic flow. Second, the model suggested that the exfoliation of epithelial cells plays an important functional role. The holes left by extruded epithelial cells determine the passive permeability of the tissue. The possibility that cell shedding may be under hormonal control (10) suggests a mechanism whereby the animal may control intestinal permeability. Third, the intestine may present a highly selective barrier despite the fact that large holes are produced by cell shedding. The holes are transient and occupy less than one-thousandth the area of the mucosal surface. Cytotoxic substances in foodstuffs, e.g. mercury, lead, cadmium, etc. having high affinity for cellular proteins would rapidly bind to the epithelial cells. The proportion diffusing into the holes left by the cells would be very small because, under

physiological conditions, the serosal side is positive and, therefore, the electro-osmotic flow through the passive channel should be in the serosal to mucosal direction. Thus practically all the toxin would become bound to the epithelial cells. When the cells are shed, the toxin wil be excreted in the feces along with the cellular debris. The reaction of iron with mucosal cells has been observed to follow this pattern (36). Thus the exfoliation of epithelial cells allows control over passive permeability and at the same time functions as a remarkably selective barrier excluding many potentially dangerous substances.

APPENDIX

The Calculation of the Area to Path Length Ratio, $A/\Delta x$

The intestine is regarded as equivalent to a set of parallel cylindrical pores of length Δx and total cross-sectional area, A cm^2, and containing a solution of composition identical to that of the bathing solution.

(A) $A/\Delta x$ FROM THE DC ELECTRICAL RESISTANCE

The resistance (R) of a solution having an equivalent conductivity of λ is given by (Harned and Owens, 37)

$$R = \frac{1000}{c\lambda}\left(\frac{\Delta x}{A}\right) \tag{63}$$

where c is the molar concentration of electrolytes. The resistance of the ileum is 4.2 ohms (Fig. 1) when bathed by 0.15 M sodium chloride solutions for which λ at 37°C is 121 (Conway, 38). Thus the cylindrical pores determining the dc resistance of the ileum, must have an A to Δx ratio of 13 according to equation (63).

(B) $A/\Delta x$ FROM THE VALUES OF f'_{iw}

From the definition of ionic mobility (Harned and Owen, 37) the flow $(J_i)_{el}$ of any ion i of valence z_i in aqueous solution under an electrical potential field of grad ψ is given by

$$(J_i)_{el} = -z_i e_i U_i \text{ grad. } \psi \tag{64}$$

Where U_i is the ionic mobility. As stated by Helfferich (39), the flow J_i relative to the matrix of the pore results from the superposition of the flow relative to the pure liquid $(J_i)_{con}$

$$J_i = (J_i)_{el} + (J_i)_{con} \tag{65}$$

If J_v is the volume flow through the pores, $(J_i)_{con}$ is given by

$$(J_i)_{con} = c_i J_v \tag{66}$$

Adding equations (65) and (66), it follows that

$$J_i = (J_i)_{el} + (J_i)_{con} = -z_i e_i U_i \text{ grad } \psi + c_i J_v \tag{67}$$

replacing grad ψ by $\Delta\psi/\Delta x$ where $\Delta\psi$ is the voltage across the intestine expressing J_i in microequivalents per hour, J_v in ml per hr per total area A, and $\Delta\psi$ in mv, equation (67) on rearrangement gives

$$\Delta\psi = \frac{\Delta x}{A} \cdot \frac{1}{3.6\ U_i}\left(\frac{J_i}{\bar{c}_i} - J_v\right) \tag{68}$$

Since equation (68) is identical to equation (15), it follows that

$$f'_{iw} = \frac{\Delta x}{A} \cdot \frac{1}{3.6\ U_i}. \tag{73}$$

The values of the products $U_i f'_{iw}$ for sodium, potassium, and chloride are approximately 2.0×10^{-2} (see Result). This leads to a value of $A/\Delta x$ of 14.

Calculation of Electroosmotic Parameters

The zeta potential was calculated from the Smoluchowski equation

$$\zeta = \frac{v}{E'_{app}} \cdot \frac{4\pi\eta_w}{D_w} \tag{70}$$

where v (cm/sec) is the velocity of water flow, E'_{app} esu/cm the applied electrical field, D_w the dielectric constant of water, and η_w poises the viscosity of water. The value of v/E'_{app} was calculated from the relation

$$\frac{v}{E'_{app}} = \frac{Jv}{A} \cdot \frac{\Delta x}{\Delta\psi} \tag{71}$$

where J_v ml/sec is the observed volume flow, and $\Delta\psi$ esu is the potential difference across the ileum. The ratio $A/\Delta x$ was taken as 14 (see above). According to linear regression analysis, the volume flow was 0.3 ml/hr at an applied current of 1 ma (Fig. 3). 1 ma is equivalent to 4.2 mv (Fig. 1). Substituting these values in the Smoluchowski equation the zeta potential is 14 mv.

The surface charge density σ esu/cm² was calculated from the Chapman-Gouy equation which, at 25°C for uni-univalent electrolytes is (James, 40)

$$\sigma = 3.59 \times 10^4 \sqrt{I} \sinh (\epsilon/53.5) \tag{72}$$

where I is the ionic strength. A value for ϵ of 14 mv leads to a value for σ of 2980 esu/cm².

The Fraction of Sodium Ions Acting As Counterions to the Fixed Charges in the Passive Channel

Assuming that the passive channel is a hole caused by the exfoliation of an epithelial cell of area 15 μ^2 and length 20 μ, the diameter of the hole will be 4 μ and the area of

the cell surfaces lining this hole will be $\pi \times 4 \times 20 = 251\ \mu^2$ or 2.51×10^{-6} cm^2, the number of fixed charges is the area times σ or $2.51 \times 10^{-6} \times 2980$ esu $= 1.6 \times 10^7$ electronic charges.

The volume of the hole is 300 μ^3 or 300×10^{-15} liters. Assuming the hole is filled with 0.15 M NaCl, the total number of sodium ions in the hole will be the molarity times Avogadro's number times the volume or $0.15 \times 6.02 \times 10^{23} \times 300 \times 10^{-15} = 2.7 \times 10^{10}$. Hence the percentage of sodium ions in the hole acting as counterions is $(1.6 \times 10^7/2.7 \times 10^{10})\ 100 = 0.06\%$.

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