ION AND WATER FLUXES IN THE ILEUM OF RATS*

BY PETER F. CURRAN‡ AND A. K. SOLOMON

(From the Biophysical Laboratory of Harvard Medical School, Boston)

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ABSTRACT

Studies have been carried out on the movement of salt and water across the small intestine of the rat. Segments of the ileum of anesthetized rats have been perfused *in vivo* with unbuffered NaCl solutions or isotonic solutions of NaCl and mannitol. Kinetic analysis of movements of Na^{24} and Cl^{36} has permitted determination of the efflux and influx of Na and Cl. Net water absorption has been measured using hemoglobin as a reference substance.

Water was found to move freely in response to gradients of osmotic pressure. Net water flux from isotonic solutions with varying NaCl concentration was directly dependent on net solute flux. The amount of water absorbed was equivalent to the amount required to maintain the absorbed solute at isotonic concentration. These results have been interpreted as indicating that water movement is a passive process depending on gradients of water activity and on the rate of absorption of solute.

The effluxes of Na and Cl are linear functions of concentration in the lumen, but both ions are actively transported by the ileum according to the criterion of Ussing (*Acta Physiol. Scand.*, 1949, **19**, 43). The electrical potential difference between the lumen and plasma has been interpreted as a diffusion potential slightly modified by the excess of active Cl flux over active Na flux.

The physical properties of the epithelial membrane indicate that it is equivalent to a membrane having negatively charged uniform right circular pores of 36 Å radius occupying 0.001 per cent of the surface area.

It has long been recognized that the intestinal epithelium is permeable in both directions to dissolved substances and to water. Goldschmidt and Dayton (1) showed that NaCl entered the colon of dogs from the blood, and a number of investigators have observed that water moves relatively freely, since it is absorbed rapidly from hypotonic solutions placed in the intestine but enters hypertonic solutions. Visscher *et al.* (2, 3) using radioactive Na and Cl and D_2O as tracers confirmed and extended these earlier results. More recently, absorption of NaCl and water has been studied by Bucher, Anderson, and Robinson (4), D'Agostino, Leadbetter, and Schwartz (5), Budolfsen (6), Goldman *et al.* (7), and Wilson (8).

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‡ Public Health Service Research Fellow of the National Heart Institute.

The present experiments were undertaken in an effort to clarify the separate roles played by active processes and passive phenomena in intestinal absorption. Studies have been carried out on the mechanisms of Na and Cl absorption, particularly as related to net water absorption. Segments of the ileum of anesthetized rats were perfused *in vivo* with solutions of varying concentrations of NaCl to which Na^{24} and Cl^{36} had been added. Measurements were made of the efflux, influx, and net flux of Na and Cl and the net flux of water.

Experimental Methods

Male albino rats weighing 250 to 450 gm. were fasted overnight and then anesthetized with nembutal (7 mg. per 100 gm.). Additional anesthetic was injected as needed during the course of the experiment. The trachea was exposed and cannulated in order to prevent the development of respiratory difficulty. The abdomen was opened with a midline incision, and the distal 15 to 20 cm. of the ileum exposed. A segment no more than 5 cm. from the ileocecal valve and about 10 cm. in length was cannulated at both ends with small glass tubes. The segment was then thoroughly washed with isotonic NaCl warmed to 37°C.

During the perfusion, the intestine was kept outside of the body in order to avoid kinking and impedance of flow; it was covered at all times with paper tissues kept moist with saline. The whole animal was placed in a specially constructed constant temperature box to maintain the segment at body temperature. The box was equipped with a thermostat and a relay operating a 60 watt electric lamp which served as the heating unit. The temperature within the box was maintained at 37° ± 0.2°C. except for short periods during the changing of perfusing solutions when it occasionally dropped as low as 34°C. At the conclusion of the experiment, which usually lasted 2 hours, the appearance of the intestine and of the blood flow to the perfused segment was generally good.

Two types of perfusion technique were used. In the majority of experiments there was a constant slow flow of perfusing solution from a small condenser, which served as a reservoir, outside the temperature control box. Water from a constant temperature bath was pumped through the jacket of the condenser to warm the perfusion fluid. Measurements showed that with the water bath at 38.5°C., the temperature of the perfusate entering the proximal cannula was 37° ± 0.5°C. The perfusate passed from the condenser through a length of 26 gauge stainless steel tubing into the proximal cannula. The tubing, because of its small radius, offered a high resistance to flow. Consequently, the fluid flowed through the intestine slowly (0.1 to 0.4 ml. per minute) with little distention pressure. 1 to 2 ml. samples of the effluent solution were collected for analysis.

In the second type of experiment, a peristaltic action pump (American Instrument Company) capable of flow rates as low as 0.2 ml. per minute circulated solution from a reservoir through the lumen and back to the reservoir. Samples were periodically removed from the reservoir for analysis. In these experiments, temperature was not controlled.

The perfusing solution was usually an unbuffered NaCl solution at a concentration[1]

[1] The symbol, mM, is used to represent quantity of a substance in millimols.

of 150 mM/liter or a solution of NaCl and mannitol with a total osmolar concentration of 300 m.osM/liter, though in some cases hypertonic or hypotonic NaCl solutions were used. Human hemoglobin was used as an indicator of water movements; a small amount was added to bring the hemoglobin concentration to approximately 3×10^{-5} M/liter. Trace amounts of Na^{24} Cl and in some cases $NaCl^{36}$ were added to the perfusate (isotopes obtained from Atomic Energy Commission). A typical experiment consisted of four 30 minute periods, during each of which a different perfusing solution was used. Five samples of effluent solutions were collected during each period. The first sample was usually discarded and the rest were each analyzed for the concentration of Na, Na^{24}, Cl, Cl^{36}, and hemoglobin. Similar analyses were carried out on the original solutions. Flow rate was measured for each collected effluent sample.

In order to show that hemoglobin is acceptable as an indicator of net volume changes, it is necessary to prove that hemoglobin is neither released nor absorbed by the intestine under present experimental conditions. The hemoglobin concentration of the samples was determined, after appropriate dilution, from the optical density measured at a wave length of 416 mμ on a Beckman model B spectrophotometer (accuracy ±1.2 per cent).[2] To show that no hemoglobin was released from the intestinal segment, the intestine was perfused with isotonic NaCl containing no hemoglobin. Optical density measurements at 416 mμ were identical before and after perfusion (O.D. before 0.000; O.D. after 0.002). To show that no hemoglobin was absorbed by the intestine, five experiments were carried out in which the segment was perfused with isotonic NaCl containing hemoglobin. Recovery of the total initial amount of hemoglobin was checked by comparison of the optical density of the collected perfusate with the optical density of a solution containing an amount of hemoglobin equal to the amount present in the solution before perfusion (average recovery 99.7 per cent). Studies were also carried out to check that NaCl and mannitol were without effect on the optical density of hemoglobin, and that the state of oxygenation of the hemoglobin did not change with passage through the intestine. These control experiments confirmed the validity of the use of hemoglobin as an indicator for water movements.

In recirculation experiments when small volumes (0.1 ml.) were obtained for analysis, optical density was measured at a wave length of 419 mμ on the microcolorimeter designed by Solomon and Caton (9). Control experiments indicated that the results of these determinations were identical with those obtained with the Beckman instrument at 416 mμ.

Na concentration was measured to an accuracy of ±1.5 per cent on the modified flame photometer described by Solomon and Caton (10). The gamma rays from Na^{24} were measured in solution in a well type scintillation counter which is free from dead time and self-absorption corrections. All counts were made to a probable error of less than 1 per cent. The total volume collected was determined from measurement of the total radioactivity of the collected solution, as compared with the radioactivity in a measured aliquot of this solution. This method yielded results accurate to ±1.5 per cent.

[2] Accuracy is given as the standard deviation of 10 replicates unless otherwise stated.

Cl concentration was determined by a modification of the method of Schales and Schales (11). HNO_3–Na citrate buffer (pH 1.5) was added to the test solution and titration carried out with 0.025 N $Hg(NO_3)_2$ using diphenyl carbazone as an indicator. The accuracy was ±1.0 per cent. Cl^{36} was measured to an accuracy of ±1.8 per cent in a Robinson windowless proportional flow counter (12) using the method of Hunter and Commerford (13) for preparing samples. Corrections were made for self-absorption. Mannitol was determined by measuring the formic acid produced by periodate oxidation according to the method of MacFadyen (14) (accuracy ±1.9 per cent). The oxidation was carried out by the method of Karnovsky and Brumm (15). In some cases, measurements of total solute concentration were made by determining freezing point depression using the American Instrument Company-Bowman apparatus.

Measurements of the electrical potential difference between the solution in the lumen and the surface of the perfused segment were made with a pair of calomel electrodes using a Keithley model 200 B direct current electrometer. A length of thin polyethylene tubing filled with 1 M/liter KCl–4 per cent agar served as a bridge; one end was sealed into the distal cannula and the other end was placed in a beaker of 1 M/liter KCl containing a calomel electrode. A similar agar bridge was placed on the surface of the intestine and connected in the same manner to the other calomel electrode. Occasionally, the second bridge was placed in the peritoneal cavity but not in contact with the perfused segment. This procedure did not affect the potential measurement significantly.

Mathematical Treatment of Data

Net Water Flux.—

The net efflux of water from the lumen can be calculated directly from changes in the optical density of the hemoglobin solutions.

$$\Phi_n^w = \frac{(v_{po} - v_p)60}{st} = \frac{60v_p}{st}[(D/D_o) - 1] \quad (1)$$

in which Φ_n^w = net efflux of water (ml./hr. cm.), t = time of collection (minutes), s = length of intestine perfused (cm.), v_p = volume collected (ml.), D = optical density, and the subscript o denotes initial conditions. A negative net flux indicates entrance of water into the lumen.

Influx and Efflux of Na and Cl.—

The treatment will be carried out for the case of Na; identical equations apply to Cl fluxes. It has been assumed that Na^{24} is handled by the intestine in the same manner as Na^{23}, and that the Na^{24} movement can be described by simple two compartment kinetics. The fluid in the lumen has been considered as one compartment and the plasma as the other. The validity of the two compartment analysis will be discussed in a subsequent section.

An isolated segment of intestine with fluid moving through it at a constant

rate has been taken as the model for mathematical treatment. The following symbols will be used in addition to those already defined.

$[Na]_p$ = concentration of Na in the lumen.
$(Na)_p$ = total amount of Na in the lumen.
$[Na]_q$ = concentration of Na in the plasma.
P = total amount of Na^{24} in the lumen (c.p.m.).
p = concentration of Na^{24} in the lumen (c.p.m./ml.).
$\bar{p}$ = specific activity of Na^{24} in the lumen (c.p.m./mM).
$\bar{q}$ = specific activity of Na^{24} in plasma.
Φ_e^{Na} = Na efflux (mM/min.); the subscript i is used for influx, e for efflux, and n for net efflux.
k_1 = rate constant for Na efflux (ml./min.)
k_{-1} = rate constant for Na influx (ml./min.)

In a simple two compartment system, Equation 2 describes the change in amount of Na^{24} in the lumen.

$$dP/dt = -\Phi_e^{Na}\bar{p} + \Phi_i^{Na}\bar{q} \tag{2}$$

Measurements of plasma specific activity at the conclusion of several experiments, when it has reached its maximum value, have indicated that $\bar{q}$ is negligible with respect to $\bar{p}$. Equation 2 can then be simplified by setting $\bar{q} = 0$ and using the identities $p = \bar{p}[Na]_p$ and $P = pv_p$.

$$d(v_p p)/dt = \Phi_e^{Na} p/[Na]_p \tag{3}$$

If Na efflux is proportional to Na concentration in the lumen, $\Phi_e^{Na} = k_1[Na]_p$. This relation assumes that the electrial potential difference across the membrane is constant and that the effect of the potential on efflux is included in k_1. The validity of this assumption is discussed below. Substituting this relation into Equation 3 and carrying out the differentiation yields

$$dp/dt = -[(dv_p/dt) + k_1]p/v_p \tag{4}$$

Changes in volume have been assumed to be linear with time over the course of an experiment, as given by Equation 5

$$v_p = v_{po}(1 - \lambda t) \tag{5}$$

The change with time of the amount of Na in the volume v_p is given by Equation 6 which is the analogue of Equation 2.

$$d(Na)_p/dt = -k_1[Na]_p + k_{-1}[Na]_q \tag{6}$$

Since the plasma Na concentration is assumed to be constant, the Na influx ($k_{-1}[Na]_q$) will be assumed to be constant and equal to Φ_i^{Na}. The validity of this

assumption will be discussed in a subsequent section. Using the relation $(Na)_p = [Na]_p v_p$, Equation 6 becomes

$$d[Na]_p/dt = \frac{-[(dv_p/dt) + k_1][Na]_p + \Phi_i^{Na}}{v_p} \tag{7}$$

Equations 4, 5, and 7 define the movements of Na in the present system. They may be solved to yield values for k_1 and Φ_i^{Na}. Using Equation 5, Equation 4 becomes

$$dp/dt = \frac{(v_{po}\lambda - k_1)p}{v_{po}(1 - \lambda t)} \tag{8}$$

which has the solution

$$\ln(p/p_o) = [(k_1/v_{po}\lambda) - 1]\ln(1 - \lambda t) \tag{9}$$

in which p_o = initial concentration of Na^{24}. Solving Equation 9 for k_1 yields

$$k_1 = v_{po}\lambda\left[\frac{\ln(p/p_o)}{\ln(1 - \lambda t)} + 1\right] \tag{10}$$

All the quantities on the right side of this equations can be obtained from experimental measurements. From Equation 5

$$v_{po}\lambda = [(v_{po}/v_p) - 1]v_p/t = \frac{v_p}{t}\left[\frac{D}{D_o} - 1\right] \tag{11}$$

Finally using Equation 11, Equation 10 may be written

$$k_1 = \frac{v_p}{t}\left[\frac{D}{D_o} - 1\right]\left[\frac{\ln(p/p_o)}{\ln(D_o/D)} + 1\right] \tag{12}$$

from which k_1 may be calculated directly.
Using Equation 5, Equation 7 becomes

$$d[Na]_p/dt = \frac{[(v_{po}\lambda - k_1)[Na]_p + \Phi_i^{Na}]}{v_{po}(1 - \lambda t)} \tag{13}$$

which has the solution

$$[Na]_p = \frac{-\Phi_i^{Na}}{v_{po}\lambda - k_1} + \left[[Na]_{po} + \frac{\Phi_i^{Na}}{v_{po}\lambda - k_1}\right](1 - \lambda t)^{\alpha} \tag{14}$$

in which $\alpha = (k_1/v_{po}\lambda) - 1$.
Solving Equation 14 for Φ_i^{Na} yields

$$\Phi_i^{Na} = (v_{po}\lambda - k_1)\left[\frac{[Na]_{po}(1 - \lambda t)^{\alpha} - [Na]_p}{1 - (1 - \lambda t)^{\alpha}}\right] \tag{15}$$

k_1 is given by Equation 12, and the other quantities in Equation 15 are all related to measured quantities.

From Equation 10, and the definition of α

$$(1 - \lambda t)^{\alpha} = p/p_o \tag{16}$$

Using Equations 11 and 16 Equation 15 becomes

$$\Phi_i^{Na} = \frac{\left\{k_1 - \frac{v_p}{t}\left[\frac{D}{D_o} - 1\right]\right\}\{[Na]_{po}(p/p_o) - [Na]_p\}}{(p/p_o) - 1} \tag{17}$$

Since Na efflux is given by $k_1[Na]_p$, and $[Na]_p$ is a function of time, integration is necessary in order to obtain the Na efflux.

$$\Phi_e^{Na} = \frac{1}{t}\int_0^t k_1[Na]_p\, dt \tag{18}$$

The integration may be carried out over the total time of collection since this is equivalent to summing the efflux for many small volumes of solution integrated over the time that the volume spends in the intestine. By definition,

$$\overline{[Na]_p} = \frac{1}{t}\int_0^t [Na]_p\, dt \tag{19}$$

in which $\overline{[Na]_p}$ = time average Na concentration in the lumen. Therefore,

$$\Phi_e^{Na} = k_1\overline{[Na]_p} \tag{20}$$

The net Na flux is given by

$$\Phi_n^{Na} = \Phi_e^{Na} - \Phi_i^{Na} \tag{21}$$

In the present experiments, calculations for a number of examples have shown that the value of $\overline{[Na]_p}$ obtained from the integration of Equation 19, using Equation 14, is closely equal to the arithmetical mean value of $[Na]_p$. Consequently the mean value has been used in the remaining calculations. In the final results, all fluxes have been expressed in terms of mM/hr. cm. for solutes and in ml./hr. cm. for water.

The validity of these equations has been tested in experiments in which a given volume of solution has been recirculated through the intestine. In this way changes due to absorption can be followed as functions of time. Equations 5 and 9 predict respectively that a plot of v_p/v_{po} against time and a plot of ln p/p_o against $\ln(1 - \lambda t)$ should be straight lines. These plots for an experiment lasting 2 hours are shown in Fig. 1. The linear relations observed indicate that the above equations describe the system adequately for a 2 hour period which was characteristic of most of the experiments performed.

The interpretation of results which follows rests on the validity of the assumption that only two compartments are effectively involved in the transport processes. It is necessary, therefore, to examine this assumption in some detail.

Anatomically, it is likely that a third compartment exists. Transport of substances probably takes place, at least in part, through the epithelial cells of the mucosa, and these cells could constitute a third compartment in addition to the lumen and the plasma. However, if this compartment contains a small amount of Na relative to the other two, it will have little effect on the kinetics of transport between the two larger compartments. Further, if this third compartment comes into rapid equilibrium with either of the two compartments it may be considered a part of that compartment, and the transport system will have only one effective barrier between the lumen and the plasma. Likewise if the transport into the third compartment is slow relative to the other com-

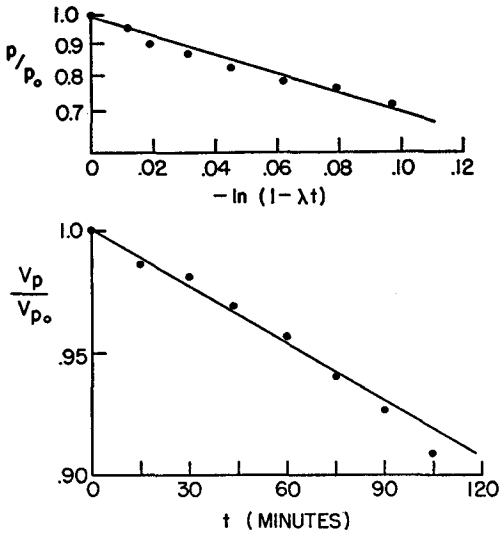

FIG. 1. Fit of experimental data to theoretical curve.

partments, it will not affect the kinetics appreciably. The validity of Equation 9, as shown in Fig. 1, indicates that the system can be described by two compartment kinetics when flux from lumen to blood is considered.

An estimate of the maximal size of the third compartment has been obtained from a determination of the total amount of Na^{24} remaining in the perfused segment of intestine at the conclusion of an experiment, as measured by direct counting of the tissue. This total comprises contributions of muscle, extracellular fluid, and mucosal cells. An estimate of muscle and extracellular fluid Na^{24} may be obtained from the amount of radioactivity present in an adjacent nonperfused segment. The remaining activity, assumed to be entirely in the mucosal cells (the third compartment), was found in three experiments to average 2.1 per cent of the total amount of Na^{24} transported across the mucosa. These findings indicate that the third compartment cannot contain a large amount

of Na^{24}, unless it fills in a period long compared with 2 hours. In agreement with this observation, Visscher *et al.* (3) have found that only 5 to 10 per cent of the D_2O leaving the small intestine of dogs remains in the mucosa. Benson *et al.* (16) have found similar results in the rat small intestine. After 18 minutes only 6.5 per cent of an administered dose of D_2O remains in the lumen and in the wall of the small intestine while the rest of the D_2O has been transported into the blood stream. Thus, these measurements support the analysis of Na transport on the basis of a two compartment system.

In the above treatment, the influx into the lumen from the plasma has been assumed to be constant and independent of the concentration of ion in the lumen. As will be shown in Fig. 7 (discussed below), this assumption is valid for Cl at all concentrations and for Na at concentrations below 100 mM/liter. Above this concentration, Na influx increases with increasing concentration. However, in this concentration range, the Na concentration does not change by more than 5 mM/liter during a perfusion period. Such a change would cause only a 3 per cent change in Na influx, and it is reasonable to assume that the influx is constant.

The assumption that the electrical potential difference across the membrane was independent of NaCl concentration in the lumen, is also not absolutely correct. A consideration of the magnitude of this effect indicates that the change in potential resulting from a change in NaCl concentration of 10 mM/liter (a change greater than that observed in any experiment) would cause a variation of less than 3 per cent in the efflux of either Na or Cl.

The fluxes measured in these experiments were quite variable from animal to animal, an observation similar to that made by Visscher *et al.* (2, 3) in the small intestine of the dog. Furthermore, there was often a considerable variation in flux from one period to another in the same animal. Consequently, the following averaging procedure has been used to express the results. In each animal, four separate determinations of flux were made with each solution perfused through the intestine; these were averaged to give a single value of the fluxes for the particular solution. When the dependence of the fluxes on concentration was studied, fluxes measured at similar concentrations in different animals were averaged.

RESULTS

Response of Net Water Flux to Osmotic Pressure Gradients.—

In three experiments, the intestine was perfused with NaCl solutions of varying tonicity. The results of a typical experiment are shown in Fig. 2. Water is rapidly absorbed from a hypotonic solution but enters a hypertonic solution, indicating that water movements are, at least in part, controlled by the gradient of water activity across the membrane. This finding indicates that the intestine is relatively freely permeable to water in response to osmotic gradients.

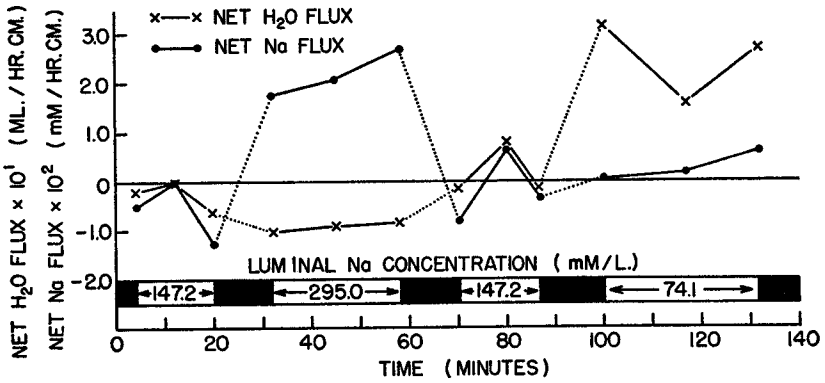

FIG. 2. Influence of osmotic gradients on net water flux. Since no mannitol was added to the perfusing fluid, the tonicity is proportional to the NaCl concentration.

TABLE I

Net Na and Net Water Flux across the Ileum When Perfused with 150 mM/Liter NaCl

Net Na flux × 10^2	Net H_2O flux
mM/hr. cm.	*ml./hr. cm.*
5.36	0.28
2.70	0.22
1.92	0.12
1.58	0.14
1.20	0.11
1.08	0.08
0.91	0.07
0.82	0.07
0.58	0.02
0.48	0.09
0.41	0.04
0.30	0.01
0.19	0.03
−0.26	−0.01
−0.56	−0.02
−0.84	−0.11
−1.28	−0.06
−2.46	−0.08
−3.25	−0.12

Linkage of Net Water Flux and Net Solute Flux.—

The results of individual net flux determinations in three experiments in which the segment was perfused with solutions of 150 mM/liter NaCl are given in Table I. A correlation coefficient calculated from the data shows that the net Na flux is significantly correlated with the net water flux ($p < 0.01$).

The movement of these substances across the intestinal wall must be governed by one or more of the following processes: (*a*) a transport mechanism in the intestinal membrane moves both water and NaCl independently and separately; (*b*) the transport mechanism moves water and NaCl follows as the result of water movement; (*c*) the transport mechanism moves NaCl, and water follows as a result of solute movement. These alternatives are mutually exclusive since if the movement can be shown to be governed entirely by either process (*b*) or (*c*), it cannot be governed by process (*a*) or a combination of (*a*) with either of the others. At this stage, we are not concerned with the distinction between active and passive transport, but rather between dependent and independent processes.

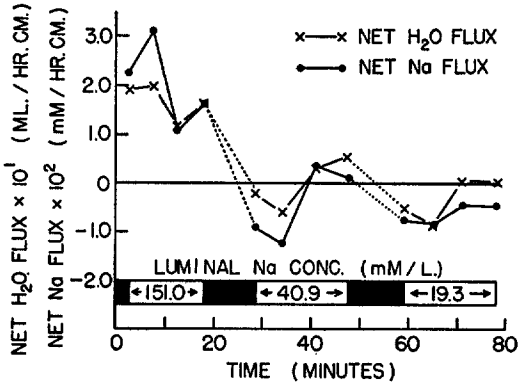

FIG. 3. Change in net water flux resulting from a change in net Na flux. The activity of water is kept approximately constant in each of the four solutions used, by replacing the NaCl with mannitol.

To test this point, nine experiments were carried out in which part of the NaCl in the perfusing solution was replaced with mannitol to provide perfusing solutions of constant tonicity but varying NaCl concentrations. Fig. 3 shows the results of a typical experiment in which three different concentrations of NaCl were employed in a single animal. It can be seen that the Na flux is dependent on the NaCl concentration in the lumen, and that the water flux follows the Na flux. These observations would support the conclusion that either process (*c*) or a combination of (*a*) and (*c*) is the operative mechanism.

The choice between these two alternatives can be made from a determination of the exact relationship between salt and water flux. The net water fluxes found in this same series of experiments are plotted in Fig. 4 against the corresponding net Na fluxes. The linear relation between these two variables has been determined by the method of least squares yielding the following equation which has been plotted as the full line in Fig. 4.

$$\Phi_n^w = (5.33 \pm 0.55)\Phi_n^{Na} + (0.04 \pm 0.01) \text{ [ml./hr. cm.]} \quad (22)$$

If the water transported is exactly equivalent to the water required to maintain the transported NaCl at an isotonic concentration, the relationship between these variables would be

$$\Phi_n^w = 6.67\,\Phi_n^{Na}\ [\text{ml./hr. cm.}] \tag{23}$$

The positive intercept of the experimental line with the y axis indicates that water is still being absorbed in the absence of net Na transport. This, together with the different slope could be ascribed either to a separate mechanism for water absorption or to the absorption of solutes other than NaCl.

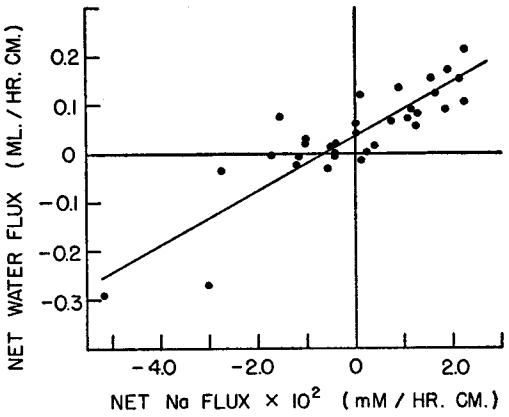

FIG. 4. Relation between net Na flux and net water flux. Each point represents an average of four separate flux measurements from a single perfusing solution in one animal. The line was determined by the method of least squares.

To determine which alternative is correct, both the net flux of Na and of mannitol, which is the only other solute present in the lumen in appreciable amount, were determined in a series of four experiments. In order to check that no osmotically important amount of solute enters the lumen during the course of perfusion, freezing point depressions were measured before and after perfusion with isotonic solutions in six experimental periods in two animals; no change was found. The movement of one equivalent of Na actually results in the movement of two osmotic equivalents of solute, since electroneutrality must be preserved. Thus the movement of one equivalent of mannitol is only one-half as effective in moving water osmotically as one equivalent of Na, and the expected relation becomes

$$\Phi_n^w = 6.67[\Phi_n^{Na} + 1/2\Phi_n^m] \tag{24}$$

in which Φ_n^m = net mannitol flux (mM/hr. cm.). The results of these experiments are shown in Fig. 5. The line drawn through the points was determined by the method of least squares and is given by

$$\Phi_n^w = (6.17 \pm 0.66)(\Phi_n^{Na} + 1/2\Phi_n^m) + (0.001 \pm 0.008) \tag{25}$$

which is in good agreement with the expected relation. It is concluded that process (*c*) is operative, and that the transport of water is a passive process, depending entirely on the absorption of dissolved substances, and on the gradient of water activity.

The apparent slight difference in slopes of the expected and observed lines might suggest that water movement lags somewhat behind the movement of Na. Such a result would not be entirely unexpected since Visscher and Roepke (17) have found that isotonic solutions become slightly hypotonic during absorption from the small intestine of dogs indicating that salt is absorbed more rapidly than water. A lag would also be in agreement with the concept that water flux follows solute flux. If the roles were reversed, water would be expected to move

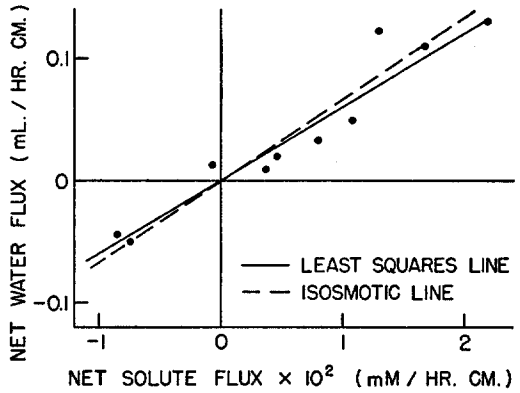

FIG. 5. Relation between net solute flux (net Na flux + ½ net mannitol flux) and net water flux. The solid line has been drawn through the points by the method of least squares; the broken line is the expected relation for transport of solute in isotonic concentration.

more rapidly than salt, and the slope of the experimental line might be greater than 6.67.

The Efflux and Influx of Na and Cl.—

The dependence of Na and Cl efflux on concentration in the lumen is shown in Fig. 6. For both ions, the efflux is a linear function of concentration in the lumen. Such a relation would be expected for a passive diffusion process, but its occurrence cannot be taken as evidence that no active processes are involved.

Influx into the lumen would be expected to be independent of concentration in the lumen, since the influx takes place from a reservoir of constant concentration (the plasma). In general, the passive diffusion of a substance in one direction across a membrane is not dependent on the concentration on the other side of the membrane. In the case of Cl, as shown in Fig. 7, the influx of Cl does indeed appear to be independent of luminal concentration. Such a situation does not, however, obtain in the case of Na. No adequate explanation exists for

the increase in Na influx with increases of Na concentration above 100 mM/liter, though it may be that the measurement of Na influx is complicated by the secretion of intestinal glands.

Comparison of the efflux and influx relationships indicates that net absorption of Na can take place from solutions as dilute as 55 mM Na/liter, and that Cl

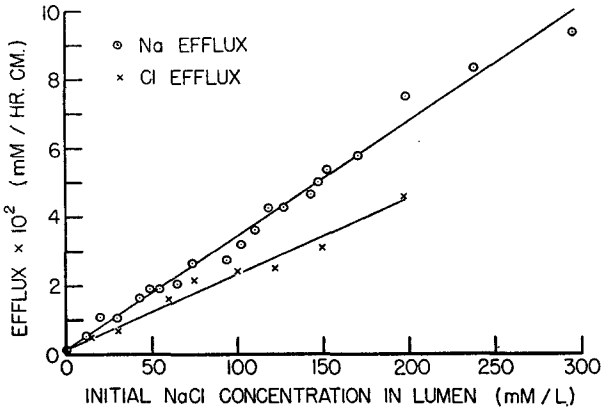

FIG. 6. Efflux of Na and of Cl from the solution in the lumen as a function of NaCl concentration. The lines were determined by the method of least squares.

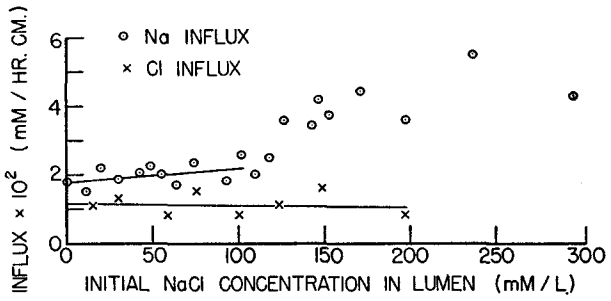

FIG. 7. Influx of Na and of Cl into the lumen as a function of NaCl concentration in the lumen. The lines were determined by the method of least squares. For Cl influx, all points were used; for Na influx only the points between NaCl concentrations of 0 and 100 mM/liter were used.

can be absorbed from a solution of 45 mM Cl/liter. Since the mean values of Na and Cl concentration in the plasma, determined at the conclusion of five experiments, were 136.0 mM/liter and 110.0 mM/liter respectively, both ions can be absorbed against appreciable concentration gradients.

Relation of Ion Fluxes to Potential Difference.—

Ussing (18) has shown that the transport of an ion may be defined as passive if, in the absence of a temperature gradient across the membrane, the ratio of

efflux to influx satisfies the following equation, which has been derived from that given by Ussing on the further assumption that the activity coefficient in the plasma is the same as that in the lumen.

$$\frac{\Phi_e}{\Phi_i} = \frac{c_1}{c_2} e^{zF(\psi_1-\psi_2)/RT} \left(\frac{a_{w1}}{a_{w2}}\right)^{G/g_w} \tag{26}$$

The subscripts 1 and 2 denote the solutions in the lumen and plasma respectively. c is the concentration of the solute, and a_w the activity of the water, ψ is the electrical potential, z the ionic charge, F, the Faraday, R, the gas constant, and T, the absolute temperature. Ussing defines G as "the friction between ion and water at unit velocity and g_w as the corresponding value for a water molecule." With respect to the solvent, it has been shown (Fig. 5) that under the present experimental conditions there is no net flow of water when there is no net flow of solute. Consequently, the activity of water, which includes osmotic and hydrostatic contributions, may be taken as equal in lumen and plasma, and Equation 26 reduces to

$$\frac{\Phi_e}{\Phi_i} = \frac{c_1}{c_2} e^{zF(\psi_1-\psi_2)/RT} \tag{27}$$

Since the concentration of Na and Cl in lumen and plasma has been measured, it is only necessary to determine the potential difference in order to calculate the expected flux ratio according to Equation 27.

Measurements of potential were made in both recirculation and constant infusion experiments. In the former, measurements at luminal concentrations of 146 and 163 mM Na/liter gave zero potential differences [$(\psi_1 - \psi_2) = -0.6 \pm 0.8$ mv. and -0.3 ± 0.9mv. respectively]. The potentials remained constant within experimental error for a period of at least 100 minutes. The electrodes were placed in the lumen and on the surface of the segment of perfused intestine. However, the significant potential difference is that between the solution in the lumen and the plasma. Consequently, it is necessary to show that the potential difference between plasma and surface of the perfused segment may be neglected. Since the electrometer used to measure the potential draws a grid current of less than 5×10^{-14} amperes in the input stage, the resistance of the additional membranes between plasma and segment surfaces does not cause a significant potential drop. Furthermore, it is assumed that there are no bioelectric potentials generated between plasma and surface.

Potential measurements, with simultaneous flux measurements, were also made at different NaCl concentrations in the same animal using the constant infusion technique described above. The results of five experiments have been averaged and are given in Table II. Column four gives the calculated flux ratios on the basis of Equation 27. The difference between these calculated flux ratios and the ones actually observed, given in column five, is interpreted to mean that the flux of Na is active according to Ussing's criterion.

Since the measured potential differences showed in all cases that the lumen

was either positive with respect to the plasma, or was equal to the plasma potential within 2 mv., simultaneous measurements of Cl flux were not made. In Table III the second column gives the lumen to plasma concentration ratio for Cl which is to be compared with the observed flux ratio given in column three. No entry has been made for the potential difference, since its effect is only to

TABLE II

Na Flux Ratios

$[Na]_{po}$	$\frac{[Na]_{po}}{[Na]_q}$	$\Delta\psi$ (lumen–plasma)	Calculated flux ratio for passive transport $\frac{[Na]_{po}}{[Na]_q} e^{\Delta\psi F/RT}$	Observed flux ratio $\frac{\Phi^{Na}_{o}}{\Phi^{Na}_{i}}$
mM/liter		mv.		
25	0.18	+18.0	0.36	0.71
66	0.47	+15.9	0.87	1.9
83	0.59	+6.6	0.76	1.1
107	0.76	+2.7	0.85	1.1
126	0.92	+0.4	0.93	1.3
146	1.1	−0.3	1.1	1.3
163	1.2	−0.6	1.2	1.9

TABLE III

Cl Flux Ratios

$[Cl]_{po}$	$\frac{[Cl]_{po}}{[Cl]_q}$	Observed flux ratio $\frac{\Phi^{Cl}_{o}}{\Phi^{Cl}_{i}}$
mM/liter		
16	0.14	0.76
29	0.27	0.50
55	0.50	2.2
66	0.60	2.7
76	0.69	1.4
101	0.92	3.0
124	1.1	2.3
149	1.4	1.9
197	1.8	5.4

increase the discrepancy between the figures in column two and those in column three. It is concluded that Cl, as well as Na, is transported actively from lumen to plasma in the ileum of the rat.

Relation of Net Na Flux to Net Cl Flux.—

In eleven experimental periods in three animals net Na and Cl fluxes were measured simultaneously. The net Cl flux is greater than the Na flux and linearly related to it according to the following equation,

$$\Phi^{Cl}_{n} = (0.77 \pm 0.13)\Phi^{Na}_{n} + (0.81 \pm 0.05)[\text{mM/hr. cm.}] \quad (28)$$

The correlation coefficient for this relationship is excellent ($p < 0.01$). Since there is a net Cl flux when the net Na flux is zero, the observed potential difference may be ascribed in part to the charge carried by the Cl ions. The potential produced by this charge transport will be reduced by the back flow of other negatively charged ions of which bicarbonate is probably the most important.

DISCUSSION

Passive Transport of Water.—

The finding that water absorption is a passive process is in agreement with the results of some earlier investigators. McDougall and Verzar (19) concluded that water movements from isotonic solutions could be explained entirely by the movements of solutes, and Wells (20) found that the rate of water absorption from the intestine was linearly related to the hydrostatic pressure of the solution in the lumen. Goldschmidt and Dayton (1) on the basis of their studies of water movement in dog intestine, consequent to changes in the tonicity of the plasma and of the fluid in the intestinal lumen, have concluded that at least a part of the water movement must be due to passive processes.

More recently there have been suggestions that water absorption from the intestine is almost entirely an active process. Visscher *et al.* (3) have concluded that the movements of water which they observed in the small intestine of the dog during *in vivo* experiments could not be explained by simple diffusion and osmosis and have suggested that an active flow of fluid takes place during absorption. Smyth and Taylor (21) and Fisher (22) have found that the absence of glucose from the inner perfusing solution of isolated segments of rat intestine completely inhibits water absorption. In addition, Fisher found that a large osmotic pressure gradient had no effect on water absorption in the isolated intestine even in the presence of glucose. On the basis of these observations he concluded that water absorption is entirely an active process.

Visscher *et al.* (3) measured the efflux and influx of water using D_2O. Their conclusion of the active nature of water absorption was based on the observation that the ratio of water efflux to influx was not equal to the ratio of water activities on the two sides of the membrane. Subsequent to this work, Koefoed-Johnson and Ussing (23) pointed out that when water is passing through a porous membrane, the flux ratio will not, in general, be equal to the activity ratio. The difference between these two ratios for passive water movement is dependent on the structure of the membrane, particularly the size of the pores. Thus it is not possible to use the flux ratio to decide whether or not water is subject to active transport without a detailed knowledge of the structure of the membrane.

The difference between the present conclusions and those of Fisher, and Smyth and Taylor may be due to the difference between the present *in vivo* and their *in vitro* preparation of intestine. In their *in vitro* preparation, the blood supply has been removed, and as stated above, the movement of water

normally observed with glucose present in the lumen was found to be independent of an osmotic pressure gradient equivalent to about 500 cm. of H_2O. Such behavior is not characteristic of the *in vivo* preparation. In the dog, Visscher *et al.* (3) and Wells (20) have found water movement in response to osmotic and hydrostatic gradients respectively. In the rat, McDougall and Verzar (19) have observed that water movement follows the osmotic gradient when anisotonic solutions were placed in the lumen of anesthetized animals. This is in agreement with the results of the present studies, as shown in Fig. 2.

The conclusion that water movement is passive to solute movement under our experimental conditions rests upon the following observations. First, Fig. 5 shows that water movement and solute movement are linearly related, and go through zero together. The amount of water that moves across the membrane

TABLE IV

Net Water Flux across Intestinal Segments

	Animal and intestinal segment	Net water flux	
		mμl./$\overline{\text{cm.}}^2$ sec.	μM/$\overline{\text{cm.}}^2$ sec.
Present studies	Rat ileum—*in vivo*	7.6	0.42
Fisher and Parsons (26)	Rat ileum—*in vitro*	9.5	0.53
Wilson (27)	Rat ileum—*in vitro*	8.9	0.49
Fisher (22)	Rat small intestine—*in vitro*	8.4	0.47
Jervis *et al.* (28)	Rat small intestine—*in vivo*	5.1	0.28
Smyth and Taylor (21)	Rat jejunum—*in vitro*	5.3	0.29
Visscher *et al.* (3)	Dog ileum—*in vivo*	4.6	0.25
Tidball and Tidball (29)	Dog jejunum—*in vivo*	8.3	0.46

is closely equal to that required for movement of an isotonic solution. Second, when the water activity is kept approximately constant by the substitution of mannitol for NaCl, the water movement follows the solute movement, as shown in Fig. 3. Finally, when osmotic gradients are applied to the system, the water moves according to the gradient. Since this last observation is different from that made by Fisher, the disagreement in our respective conclusions may be ascribed to differences in the intestinal preparation used.

It is heartening that in other respects there is very good agreement between the results of the present experiments and those of others, particularly in the case of net water efflux. Since in the first instance, water flux may be assumed proportional to the total surface area across which the flux is measured, comparisons will be made on the basis of mean mucosal surface area. In the case of the rat, surface area has been measured by Fisher and Parsons (24); in the case of the dog by Warren (25). The present results for water flux, 0.12 ml./(hr. cm. length) in six experiments using isotonic NaCl, may be converted to 7.6×10^{-6} ml./$\overline{\text{cm.}}^2$ sec., or 0.42 μM/$\overline{\text{cm.}}^2$ sec. Table IV shows the comparison of this

result with figures calculated from the data given by other workers both in the rat and in the dog. The agreement between these several results is surprisingly good, notwithstanding the fact that there are three preparations of rat intestine, and two species included in the data presented in Table IV.

Active Transport of Na and Cl.—

Active transport of both Na and Cl by the intestine has been previously suggested by many investigators. Visscher *et al.* (3) found that Cl movements in dog intestine could not be explained by simple diffusion; Goldman *et al.* (7) arrived at similar conclusions with respect to Cl, but suggested that Na movements were passive, D'Agostino, Leadbetter, and Schwartz (5) suggested that both Na and Cl were actively transported. However, electrical potential difference was not taken into account in any of these experiments.

In the present study it has been shown from the data presented in Tables II and III that Na and Cl are each actively transported against an electrochemical potential gradient according to the definition of Ussing (18).[3] On this basis, it is possible to explain three observations on absorption which have been the concern of investigators since the time of Heidenhain. Net absorption of water from slightly hypertonic solutions takes place because the rapid absorption of the solute provides a driving force for water absorption sufficient to overcome the adverse osmotic pressure. When in further experiments, the initial tonicity of the solution in the lumen is increased, a point will be reached where solute absorption is no longer sufficient to balance the osmotic pressure effect, and water will then enter the fluid in the lumen. The absorption of an animal's own serum from its intestine can be fully explained by active solute absorption which will, in turn, result in the absorption of water. The absorption of both Na and Cl against concentration gradients, as has been shown, is in accord with the active transport of these ions.

The "Cl impoverishment" system of Visscher and his coworkers (30) can also be explained by the active transport of Na and Cl. In their experiments, an isotonic solution of NaCl and Na_2SO_4 is placed in the intestine, and the concentration of Cl is observed to fall quite rapidly, approaching zero in many cases. The intestine is only slightly permeable to sulfate ion, and as a result, water is

[3] No consideration of "exchange diffusion" has been included in the application of this criterion. Exchange diffusion cannot contribute to the net transport of an ion in the absence of both electrical and chemical potential gradients. In the case of Na, the potential difference is zero, when the luminal concentration is 136 mM/liter, equal to the plasma concentration; under these conditions the net Na efflux is equal to 1.10×10^{-2} mM/hr. cm. In the case of Cl, the potential difference is -2 mv. (plasma negative to lumen) when the lumen concentration is 110 mM/liter, equal to the plasma concentration. Under these conditions, the net Cl efflux is 1.45×10^{-2} mM/hr. cm., and transport is up a slight potential gradient.

retained in the lumen along with Na_2SO_4. Net absorption of NaCl takes place actively, as has been shown above; and NaCl will continue to move out of the intestine until an equilibrium between efflux and influx is reached, presumably limited by the availability of Cl ion. Passive water movement, controlled by active NaCl fluxes, in accord with the considerations given by Koefoed-Johnson and Ussing (23), causes the "forced fluid flow" proposed as the mechanism of intestinal absorption by Visscher and his colleagues (3). Metabolic posions have been shown by Ingraham and Visscher (31) to inhibit Cl uptake from the intestine. On the present basis this is interpreted as an effect on the active transport of solute. Consequently, transport during poisoning should be due only to free diffusion, and the concentration of Cl in the lumen would be

TABLE V

Comparison of Fluxes from Isotonic Saline in the Ileum of the Rat and the Dog

Species	Na efflux	Na influx	Cl efflux	Cl influx	Water efflux
	$\mu\mu M/\overline{cm.^2}$ sec.				$m\mu l./\overline{cm.^2}$ sec.
Rat (present studies)	3400	2355	1980	734	25.1*
Dog (3)	4410	2400	2050	757	30.0

* Calculated from the following equation derived from Equation 13 of Reference 23

$$\Phi_e^w = \frac{\Phi_n^w e^a}{e^a - 1}$$

in which

$$a = \frac{\Phi_n^w}{D_w(A/\Delta x)}$$

expected to approach the concentration in the blood, as has been observed by Ingraham and Visscher.

The development of a slight degree of hypotonicity during the absorption of isotonic NaCl solutions as observed by Visscher and Roepke (17) is in accord with the present hypothesis. This may be ascribed to the lag of net water absorption behind net solute absorption as shown in Fig. 5. Wells (20) observed that the pressure in the lumen had to be reduced below one atmosphere to stop absorption of water. This observation is also in agreement with the present hypothesis since at hydrostatic equilibrium the active absorption of solute should cause the absorption of water from isotonic solutions.

In the case of Na and Cl flux, as in the case of the water flux, there is a considerable measure of agreement between the present results and those of other workers, in this case, Visscher and his associates. Table V presents a comparison of results calculated on the basis of flux per square centimeter of

mucosal tissue. It will be seen that in every instance, notwithstanding the species difference, the agreement is good, including the case of water efflux, calculated from the present results according to the equations of Koefoed-Johnson and Ussing (23).

Calculated Physical Characteristics of Membrane Equivalent Pores.—

The present experiment results can be used to estimate the average pore size in the membrane and the role played by diffusion in the movements of Na and Cl. Using the data obtained for the diffusion of mannitol through the membrane, and the rate of flow of water under an osmotic pressure gradient (obtained in three experiments with 75 mM NaCl solutions to which mannitol was not added) the average pore radius may be estimated using Fick's and Poiseuille's laws as has been done by Pappenheimer, Renkin, and Borrero (32) for the capillaries in the cat's hind limb. From Fick's law,

$$\frac{A}{\Delta x} = \frac{\Phi_e^m}{D_m C_m} \tag{29}$$

in which A = total area available for diffusion, Δx = length of pores, Φ_e^m = efflux of mannitol (4.9×10^{-5} mM/sec.), D_m = diffusion coefficient for mannitol from International Critical Tables (9.2×10^{-6} cm.^{2}/sec. at 37°C.), and C_m = concentration of mannitol (150 mM/liter). This calculation gives a value for $A/\Delta x$ of 35.2 cm. per 10 cm. length of intestine. From Poiseuille's law, the pore radius is given by

$$r^2 = \frac{\dot{q} 8 \eta}{(A/\Delta x)\Delta P} \tag{30}$$

in which r = average radius of pores in cm. $\dot{q}$ = rate of water flow (4.1×10^{-4} ml./sec.), ΔP = pressure gradient (3.90×10^6 dynes/cm.^{2}), and η = viscosity of water (7.0×10^{-3} poise at 37°C.). Renkin (33) has given equations from which data obtained with mannitol as a probing molecule can be corrected approximately to apply when water is used as probing molecule. After making this correction, the average equivalent pore radius becomes 36 Å.

On the basis of the equations developed by Koefoed-Johnson and Ussing (Equations 19 and 21, Reference 23), the observations of Visscher *et al.* (3) lead to a calculated equivalent pore radius in the membrane of about 40 Å. This calculation gives only a rough order of magnitude for the pore radius since the conditions of the experiment do not satisfy the conditions imposed for the development of the equation used. Nonetheless, it is in good agreement with the results of the present experiments; furthermore, the radius proposed for the pores in the capillaries of the hindlimb of the cat by Pappenheimer (34) is 30 to 45 Å.

The assumptions inherent in such calculations have been discussed in detail

by Pappenheimer (32, 34) and Durbin, Frank, and Solomon (35). In addition, there are certain restrictions which apply in particular to the present estimate, which is a by-product of an investigation directed toward other ends. Thus, the membrane has been assumed impermeable to NaCl in calculating the osmotic pressure gradient; the known permeability of the membrane to NaCl will reduce the osmotic pressure gradient.

Pores of 36 Å would retain, in large measure, proteins and other large molecules, as has been observed with hemoglobin in the present experiments. Nonetheless small molecules, particularly uncharged ones, would be absorbed from the intestine by simple diffusion. This physical picture provides for the nonspecific absorption of the breakdown products of the digestive process. Pores of 36 Å radius would also account for Fisher's observation that urea, creatine, and sorbitol are all absorbed at rates proportional to their concentration gradients when water is absorbed. The absence of solute absorption in Fisher's experiments when water is not being absorbed would appear to be related to the peculiar behavior of the *in vitro* preparation with respect to water movement in response to osmotic gradients.

Given the value of $A/\Delta x$ calculated from Equation 29 and the assumption that transport from the plasma into the lumen is passive for Cl at all concentrations and for Na in the concentration range 0 to 100 mM/liter, it is possible to draw further conclusions about the equivalent physical structure of the membrane. Since the concentration gradients and fluxes are known for both ions, it is possible to calculate an effective diffusion coefficient through the pores and to compare it with the diffusion coefficient in free solution. On this basis, the diffusion coefficient of Na through the membrane is 1.55×10^{-5} cm.^{2}/sec., or 84 per cent of 1.85×10^{-5} cm.^{2}/sec. the diffusion coefficient of Na in solution at 37°C. as calculated from the diffusion coefficients of NaCl and Na_2CO_3 given in the International Critical Tables. This calculation is made on the basis of the relative amounts of NaCl and $NaHCO_3$ in the plasma using the assumption that the diffusion coefficient of $NaHCO_3$ is the same as that of Na_2CO_3. The restriction of diffusion of Na may be laid provisionally to the tortuosity of the path and the uncertain shape of the channel through which it may diffuse, though other explanations can certainly not be excluded. Considering the approximations involved in the calculations, and the effect of the charge barrier described below, the agreement of the diffusion coefficients of Na in the membrane and in free solution is good.

In the case of Cl, the calculated diffusion coefficient through the pores is 0.51×10^{-5} cm.^{2}/sec., or only 25 per cent of 2.08×10^{-5} cm.^{2}/sec., the diffusion coefficient of NaCl in free solution. The restriction of diffusion of Cl, as compared with Na, may well be attributed to the presence of a negative charge barrier in the membrane. The charge density necessary to account for this restriction may be approximated from the equations given by Teorell (36): it lies between

an upper limit of 0.64 mM/ml. and a lower limit of 0.16 mM/ml. Using an equivalent pore radius of 36 Å, and a cellular membrane thickness of 0.1 μ, estimated from the electron microscope studies of the rat intestinal mucosa given by Granger and Baker (37), the charge density is of the order of 1 charge per 100 to 390 $\bar{A}^2$ of the pore wall area, calculated on the basis of right cylinders of uniform length (0.1 μ) and radius (36 Å) and assuming a uniform distribution of charge. The fraction of the surface area occupied by pores can be estimated as 0.001 per cent or 1 pore per 4×10^7 $\bar{A}^2$.

The picture of the equivalent pore that emerges from these calculations, both in respect to pore diameter and ionic charge density, must be viewed in the light of the approximations that have been used to delineate it. It represents a physical approximation of an idealized pore based on present knowledge and

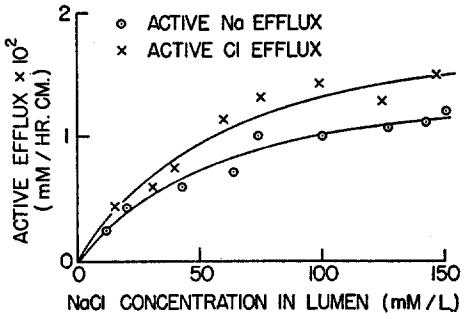

FIG. 8. Effect of NaCl concentration in the lumen on the active efflux of Na and Cl from the lumen. Circles represent Na efflux and crosses represent Cl efflux. The curves were drawn using the constants obtained from least squares analyses of Lineweaver-Burk plots.

inadequate theory. As more exact information accrues and more adequate theory is developed, the image of the physical structure of the membrane will surely clarify.

Active Transport of NaCl and Potential Differences.—

On the assumption that transport of Na and Cl from plasma to lumen is passive, Equation 27 permits calculation of the passive component of lumen to plasma flux, and hence, by subtraction, of the active component of this flux. The active efflux is shown in Fig. 8 as a function of the initial NaCl concentration in the lumen. The curves have been obtained by the method of least squares applied to a Lineweaver-Burk plot of the Na and the Cl data ([active efflux]$^{-1}$ plotted against [NaCl]$^{-1}$). The active component of efflux represents only a small fraction of the total efflux at luminal concentrations above 50 mM NaCl/liter, and hence cannot be distinguished in Fig. 6. Both curves appear to be of a saturable type, an observation which is characteristic of transport

systems assumed to be mediated by carriers. Furthermore, it would appear that the transport system differs in some essential particulars for the two ions.

The data in Fig. 8 indicate that more negatively charged ions than positively charged ones are actively transported out of the lumen. Furthermore, it has been shown that the passive influx of Na from blood into lumen is greater than the influx of Cl, which would tend to enhance the potential difference across the membrane. The Henderson diffusion equation, with the mobilities modified in accordance with the diffusion coefficients in the membrane given above, has been used as the basis for the calculation of the potential difference to be expected across the membrane. In order to compensate for the active efflux of Cl

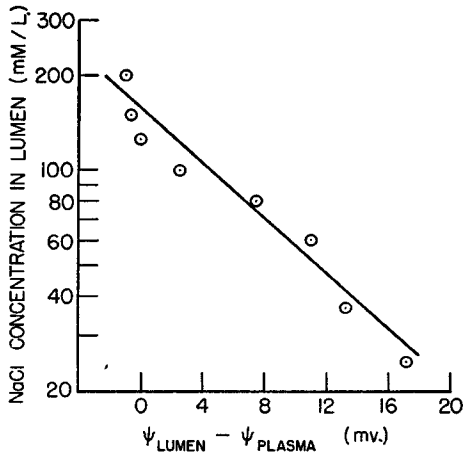

FIG. 9. Electrical potential difference across the intestine as a function of NaCl concentration in the lumen. The line has been determined from Henderson's equation as explained in the text.

(in excess of Na), the Cl mobility has been corrected by adding the excess active Cl efflux to the passive Cl efflux, and calculating a concentration dependent "effective membrane mobility." Fig. 9 shows that the theoretical potential difference is in good agreement with the experimental results. Thus it may be concluded that the potential difference across the membrane may be ascribed to a diffusion potential, modified slightly by the excess active transport of Cl in the reverse direction. The good agreement between experiment and theory shown in Fig. 9 also provides indirect evidence in support of the assumption that the potential difference between blood and peritoneal cavity may be neglected.

Fisher (22) has shown that glucose is necessary for the efflux of water from the intestine. In the present view, the requirement for glucose arises from the active nature of the NaCl transport, to which water flux is secondary. The energy production in the mucosa can be estimated from the data of Newey,

Smyth, and Whaler (38) on the conversion of radioactive glucose to CO_2 and lactic acid by an *in vitro* preparation of ileum. Calculations on the basis of these results give a value of energy production of 0.26 cal./hr. cm. length. The active component of the transport of NaCl into the plasma, under the most unfavorable conditions when the intestinal concentration is 30 mM/liter, requires a minimum supply of energy equal to 0.017 cals./hr. cm. length, or 1786 cals./mol NaCl. This represents a drain of only 6.5 per cent of the energy available. Thus there is a very large energy reserve available for the essential physiological function of water conservation by the intestine. In support of this conclusion, Fisher has found that reduction of the glucose concentration in the intestine with consequent reduction of glucose absorption to less than half his normal value, is without effect on water absorption. On the other hand, Fisher found the inhibition of glucose absorption by phlorizin poisoning to be linearly related to inhibition of water absorption. This suggests that phlorizin acts on a link in glucose metabolism which is intimately connected with NaCl transport.

SUMMARY

Experiments have been carried out to measure the flux of Na, Cl, in both directions and the net water flux across the intestinal epithelium of the rat ileum. The following major conclusions have been drawn from the experimental results. Water movement from the intestine to the blood is passive to solute movement, in this case NaCl movement. Both Na and Cl are transported actively and individually from intestine to blood. The measured potential difference may be accounted for in terms of a diffusion potential, as modified by the active components of Na and Cl flux. These conclusions form the basis for a self-consistent explanation of water absorption from the intestine which accounts for the observations made by Heidenhain, Visscher, and many subsequent investigators. The epithelial membrane may be described in physical terms as equivalent to a membrane with uniform negatively charged right circular pores of 36 Å radius, occupying 0.001 per cent of the surface area.

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