

Na, Cl, and Water Transport by Rat Colon

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ABSTRACT Segments of the colon of anesthetized rats have been perfused *in vivo* with isotonic NaCl solutions and isotonic mixtures of NaCl and mannitol. Unidirectional and net fluxes of Na and Cl and the net fluxes of water and mannitol have been measured. Net water transport was found to depend directly on the rate of net Na transport. There was no water absorption from these isotonic solutions in the absence of net solute transport, indicating that water transport in the colon is entirely a passive process. At all NaCl concentrations studied, the lumen was found to be electrically negative to the surface of the colon by 5 to 15 mv. Na fluxes both into and out of the lumen were linear functions of NaCl concentration in the lumen. Net Na absorption from lumen to plasma has been observed to take place against an electrochemical potential gradient indicating that Na is actively transported. This active Na transport has been interpreted in terms of a carrier model system. Cl transport has been found to be due almost entirely to passive diffusion.

The small intestine and the colon both function to reabsorb the NaCl and water which enter the gastrointestinal tract by secretion and by ingestion. However, the colon is able to maintain a concentration gradient of Na between lumen and plasma (1) while such a gradient does not usually develop in the small intestine unless significant amounts of polyvalent anions are placed in the lumen (2-4). Further, there is a difference in the electrical properties of these two tissues. An electrical potential difference has been observed between the lumen and the surface of both rat (5) and dog (6) colon *in vivo* when isotonic NaCl is placed in the lumen, whereas no such potential has been observed in rat (4) and dog (7) ileum under similar conditions. A potential difference is also maintained across *in vitro* preparations of toad and bullfrog large intestine bathed with the same solution on both sides (8, 9). Thus, there appear to be differences between the ion transport properties of the ileum and the colon.

The present experiments were undertaken in an effort to clarify the roles played by active and passive processes in transport of Na, Cl, and water by

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This work has been supported in part by the Atomic Energy Commission and by a research grant—A-1824(Cl)—from the National Institute of Arthritis and Metabolic Diseases, Public Health Service. Dr. Curran is a Public Health Service Research Fellow of the National Heart Institute. His present address is: Institute of Biological Chemistry, University of Copenhagen, Copenhagen.

Received for publication, May 6, 1959.

rat colon *in vivo*. Segments of colon with intact blood supply have been perfused with isotonic solutions. The unidirectional and net fluxes of Na and Cl and the net flux of water have been measured, and the relationship between net solute flux and net water flux has been examined.

Methods

Male albino rats (250 to 400 gm.) were anesthetized with nembutal (7 mg./100 gm.) and segments of colon 4 to 6 cm. in length were isolated between two cannulae for perfusion. The preparation of the animal and the method of perfusion were identical with the procedure used previously in studies on rat ileum (4). Absorption rates were determined from changes in the composition of the fluid occurring during passage through the intestine.

The perfusion fluids contained varying concentrations of NaCl and were kept isotonic by the addition of the necessary amount of mannitol. Small amounts of human hemoglobin, Na²⁴Cl, and NaCl³⁶ were added to these solutions, but no other solutes were present. The hemoglobin served as a reference substance for the determination of net water transport. Control experiments showed that, under the present conditions, hemoglobin was neither absorbed nor released during passage of fluid through the colon and its absorption spectrum was not altered. Therefore, hemoglobin serves as a dependable indicator of net water transport, and the rate of water movement can be calculated directly from changes in the optical density of the solution occurring during passage through the intestine.

The use of Na²⁴ and Cl³⁶ has permitted estimation of the unidirectional fluxes of Na and Cl across the mucosa. The kinetics of tracer movement have been analyzed on the basis of a two compartment system with the solution in the lumen considered as one compartment and the plasma as the other. The details of the flux calculation, which takes into account changes in both volume and solute concentration in the lumen, have been given by Curran and Solomon (4).

Optical density of solutions was measured, after appropriate dilution, at 412 m μ on a Beckman model B spectrometer. Na concentration was determined using the flame photometer described by Solomon and Caton (10), and Cl concentration by the method of Schales and Schales (11) as modified by Brun (12). Mannitol concentration was determined by the method of MacFadyen (13) as modified by Karnovsky and Brumm (14). Na²⁴ γ rays were measured in solution using a well type scintillation counter, and the beta radiation of Cl³⁶ was measured in a Robinson windowless proportional flow counter (15). Both isotopes were present in the solutions but could easily be assayed separately. An aluminum shield in the scintillation counter absorbed the weak beta radiation of Cl³⁶ so that Na²⁴ could be measured without interference. The samples were then stored for a week to allow the short half-life of Na²⁴ to decay completely before counting Cl³⁶. The electrical potential difference between the solution in the lumen and the surface of the colon was measured using saturated KCl-4 per cent agar bridges and calomel electrodes. A Keithley model 200B direct current electrometer was used as a detector.

RESULTS

Water Transport

The use of isotonic perfusion fluids having varying NaCl concentrations has permitted study of the relationship between net water transport and net Na transport without the complications resulting from large osmotic pressure gradients. As shown in Fig. 1, water transport from these isotonic solutions is dependent on the rate of net Na transport. A reduction in Na concentration in the lumen results in a decrease in net Na absorption which is followed by a decrease in net water absorption. This observation suggests that water absorption from the colon cannot be due entirely to an active process taking

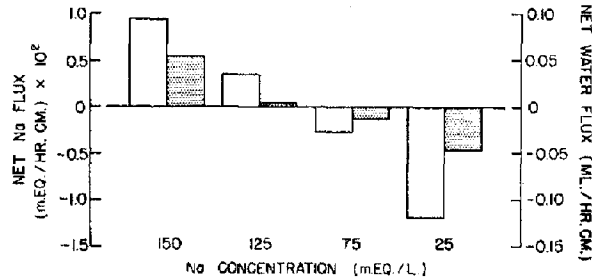


FIGURE 1. Net Na and water fluxes as a function of NaCl concentration in the lumen. The shaded blocks refer to water transport. The results for the four different concentrations were all obtained in a single experiment on the same animal.

place independently of solute transport, but does not prove that water transport is entirely passive (4).

A knowledge of the exact relationship between net water transport and total net solute transport is essential for determining whether any water is actively transported by the colon (4, 16). Under the conditions of the present experiments, the net flux of mannitol may make an appreciable contribution to total net solute transport. A net Na flux represents transfer of two osmotically active particles (to preserve electroneutrality), whereas mannitol transfer represents only one-half as much solute movement as does Na. Thus, total net solute flux is given by net Na transport plus one-half net mannitol transport. Table I summarizes the results on water and solute transport obtained in 21 experiments representing 80 determinations of Na and water fluxes and 32 determinations of mannitol fluxes. In Fig. 2, the total net solute transport is plotted against net water transport. The best straight line through

the experimental points, determined by the method of least squares, is given by Equation 1

$$\Phi_n^w = (6.15 \pm 0.90) \Phi_n^s - (0.002 \pm 0.008) \text{ [ml./hr. cm.]} \quad (1)$$

in which Φ_n^w is net water transport and Φ_n^s is net solute transport. The zero intercept of this line with the y axis indicates that there is no water absorption from these isotonic solutions in the absence of net solute transport. As discussed

TABLE 1*

Na concentration	Mannitol concentration	Φ_n^{Na}	Φ_n^M	$\Phi_n^S = \Phi_n^{Na} + 1/2\Phi_n^M$	Φ_n^w
m.eq./liter	m.eq./liter		m.eq./hr. cm. $\times 10^2$		ml./hr. cm.
30	240	-1.0	1.6	-0.2	-0.03
55	190	-0.7	1.2	-0.1	0.01
80	140	0.4	0.8	0.8	0.04
105	90	0.4	0.6	0.7	0.04
125	50	0.8	0.2	0.9	0.06
150	0	1.5	0.0	1.5	0.09

* The symbol, Φ_n , refers to net flux, and the superscripts Na, M, S, and w refer to sodium, mannitol, solute, and water respectively.

by Curran and Solomon (4), this result indicates that water transport in the colon is entirely a passive process. If no net water transport occurs between solutions of equal water activity when there is no net solute transport, water movement must be a passive process depending only on gradients of water activity, and not on any other forces.

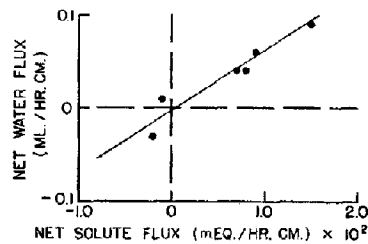


FIG. 2. Relationship between net water flux and total net solute flux (net Na flux + one-half net mannitol flux). The line through the experimental points has been determined by the method of least squares.

Na and Cl Transport

Fig. 3 shows Na fluxes into and out of the lumen as functions of NaCl concentration in the lumen. The efflux (lumen to plasma) is a linear function of this concentration over the range studied. At a NaCl concentration of 75 m.eq./liter, the influx and efflux are equal indicating zero net transport. At higher concentrations net Na transport takes place from lumen to plasma. Since the average plasma Na concentration is 136 m.eq./liter, Na can be

transported from lumen to plasma against a concentration gradient. Further, as shown in Table II, the solution in the lumen was found to be electrically negative, relative to the surface of the colon, at all NaCl concentrations studied. (This potential difference has been taken to represent the difference between the solution in the lumen and the plasma on the assumption that there are no sources of potential between the plasma and the surface.) Thus, net Na transport can take place against an electrochemical potential gradient and must, according to the definition of Rosenberg (17), be considered an

TABLE II

NaCl Concentration in lumen		Potential difference (lumen - plasma)
m.eq./liter		mv.
30		-12.4
55		-9.8
80		-7.4
105		-9.0
125		-8.5
150		-7.3

active process. As shown in Fig. 3, the influx of Na also appears to depend on Na concentration in the lumen, increasing from 1.4×10^{-2} m.eq./hr. cm. at a Na concentration of 30 m.eq./liter to 2.6×10^{-2} m.eq./hr. cm. at 150 m.eq./liter. This effect of concentration on the side of the membrane toward which a unidirectional flux is directed (the transconcentration for that flux) suggests that Na influx is not entirely the result of simple diffusion from a reservoir of constant Na concentration (the plasma).

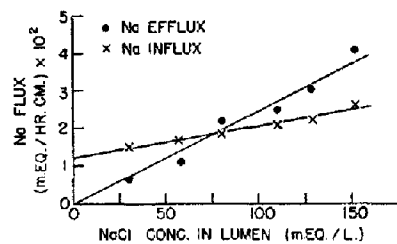


FIGURE 3. Unidirectional Na fluxes as functions of NaCl concentration in the lumen. The lines have been determined by the method of least squares.

The relationship between Cl fluxes and NaCl concentration in the lumen is shown in Fig. 4. Cl efflux is a linear function of concentration in the range studied, while influx is nearly independent of this transconcentration. The two unidirectional fluxes are equal at a Cl concentration of 70 m.eq./liter. Since the average plasma Cl concentration is 110 m.eq./liter, net Cl transport can also take place against a concentration gradient in the colon. However, the orientation of the electrical potential difference across the membrane

requires a somewhat different treatment in order to determine whether Cl is actively or passively transported. Ussing (18) has shown that an ion crossing

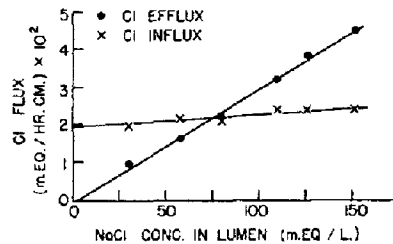


FIGURE 4. Unidirectional Cl fluxes as functions of NaCl concentration in the lumen. The lines have been determined by the method of least squares.

a membrane independently and under the influence of concentration and electrical potential gradients only should obey the following equation

$$\frac{\Phi_{12}}{\Phi_{21}} = \frac{\gamma_1 C_1}{\gamma_2 C_2} e^{zF(\psi_1 - \psi_2)/RT} \quad (2)$$

in which Φ_{12} and Φ_{21} are fluxes in the two directions across the membrane. C is concentration, γ activity coefficient, ψ the electrical potential, and the subscripts 1 and 2 denote lumen and plasma respectively. z is the charge of the ion and F , R , and T have their usual significance. In Fig. 5, the experimentally observed flux ratios for both Na and Cl are compared with the ratios expected from concentration and electrical potential gradients. The points for Cl fall quite close to the line representing equality of the observed and expected ratios, suggesting that Cl crosses the mucosa of the colon almost entirely by passive diffusion. On the other hand, the points for Na are all well above the line, indicating that the observed ratios are greater than those predicted from Equation 2, as would be expected since Na is actively transported from lumen to plasma.

The use of Equation 2 in distinguishing between active and passive transport is not unequivocal. A difference between observed and expected flux ratios could be due to bulk flow of water (8, 19), "single file diffusion" (20), or exchange diffusion (18) rather than active transport. The observation of net Na transport against an electrochemical potential gradient indicates that the observed Na ratios cannot be entirely explained by either of the last two mechanisms. Measurement of net flux is not dependent on the use of tracers as is determination of unidirectional flux. The fact that water transport is passive and dependent on net Na transport suggests that bulk water flow cannot explain the observed active Na transport. The small effect of water flow on ion movements in these experiments is further confirmed by the data

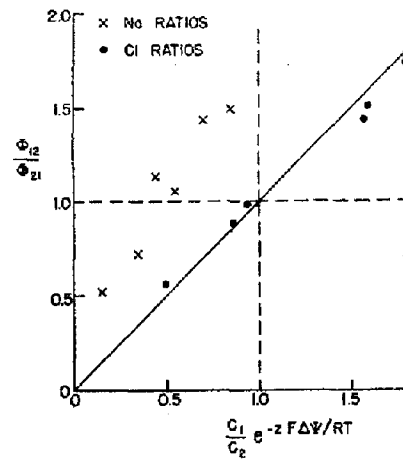


FIGURE 5. Relationship between observed and expected flux ratios for Na and Cl. $\Delta\psi = \psi_1 - \psi_2$. The solid line represents equality between observed flux ratios and those expected from concentrations and electrical potential gradients. The different points were obtained by varying NaCl concentration (C_1). When C_1 goes to zero, Φ_{12} must become zero, and Φ_{12}/Φ_{21} will go to zero for both active and passive transport.

on Cl transport. If water flow had an appreciable effect on ion transport, the observed Cl flux ratios would not be equal to those predicted from concentration and electrical gradients only.

The relationship between net Na transport and net Cl transport in the

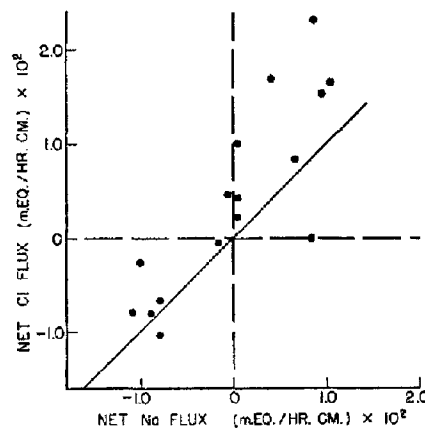


FIGURE 6. Relationship between net Na and net Cl fluxes. The solid line represents equality of the two fluxes. The points represent flux values obtained in experiments in which both Na and Cl fluxes were measured simultaneously.

colon is shown in Fig. 6 where the Cl flux is plotted as a function of the Na flux. The values were obtained in experiments in which the two fluxes were measured simultaneously in the same animal. With the exception of two

points, Cl fluxes were always greater than Na fluxes. Electrical neutrality can be maintained either by net transport of some cation other than Na from lumen to plasma or some anion other than Cl from plasma to lumen.

DISCUSSION

Passive Transport of Water

Water absorption seems to take place by the same mechanism in both ileum and colon. Water transport is entirely passive and linked closely to active solute transport; when isotonic NaCl solution is placed in the lumen, the gradient of water activity necessary for water absorption is provided by active Na transport. However, the exact processes bringing about the relationship between water and solute transport are not entirely clear. Removal of solute from the lumen by active transport could create the gradient of water activity necessary for water transport. The osmotic activity of plasma (21) and isotonic NaCl-Na₂SO₄ solutions (22) placed in the lumen of dog ileum decreases during absorption, and the resulting gradient of water activity could account for water absorption from initially isotonic solutions. However, decrease in solute concentration in the lumen cannot explain the observation that water can be absorbed from slightly hypertonic NaCl solutions in both ileum and colon (23, 24). These observations could be explained, however, if active solute transport resulted in a rise in solute concentration on the plasma side of the membrane. Such a mechanism would require solute transport at a sufficiently rapid rate to raise the solute concentration of the plasma while it remains in contact with the mucosa. Any change in concentration would tend to be counterbalanced by the continued renewal of the plasma *via* blood flow. However, the concentration gradient required in the steady state need not be large. The theoretical considerations of Cole and Meredith (25) have indicated that the magnitude of such a gradient should depend on the relative permeability of the membrane to solute and water; when the membrane is much more permeable to water than to solute, the steady state gradient may be quite small.

An estimation of possible changes in the solute concentration of the plasma supplying the mucosa can be made from knowledge of the rates of blood flow and of net solute transport. Atkinson, Parsons, and Smyth (26) have observed a blood flow in dog intestine of 45 ml./min. 100 gm. tissue. Assuming a similar rate for the rat and a weight of 30 mg./cm. length, this yields a plasma flow of approximately 1.1×10^{-4} ml./sec., equivalent to a solute flow of 1.7×10^{-5} m.eq. NaCl/sec. cm. length. The rate of net NaCl absorption in the colon is 4.0×10^{-6} m.eq./sec. cm. length. This transport represents approximately 25 per cent of the NaCl present in the plasma flowing

through the intestine, and could, therefore, result in an appreciable increase in solute concentration of the plasma while it is in contact with the mucosa. This calculation represents a minimum estimate of the proportion between solute transported and solute in the plasma supplying the mucosa since it assumes that all blood flow flowing through the intestine comes into contact with the mucosa. The actual change in plasma NaCl concentration under steady state conditions must be quite small since water is transported at a rate nearly equal to the rate necessary to maintain the absorbed solute at isotonic concentration.

Active Na Transport

The finding that Na is actively transported by rat colon whereas Cl is passively transported is in agreement with the results of studies on toad (8) and bullfrog (9) large intestine and on guinea pig cecum (8) which were carried out *in vitro* using the short-circuit technique. In toad intestine, the short-circuit current was found to be equal to net Na transport, suggesting that Na was the only ion actively transported by this tissue. In the bullfrog, the current was frequently greater than the net Na transport, suggesting active transport of another cation in the same direction as Na or an anion in the opposite direction. Recently, Cooperstein and Brockman (6) studying ion transport in dog colon *in vivo* found that Na was actively transported from lumen to plasma while Cl transport was passive, in agreement with the present findings. In some preparations, they found evidence for an active bicarbonate secretion into the lumen. Cooperstein and Hogben (9) have also found that Cl transport is passive in the bullfrog large intestine. Thus, present information suggests that a gradient of active transport properties for Na and Cl exists along the gastrointestinal tract. In stomach, Cl is actively transported from plasma to lumen while Na movement is largely passive (27). In ileum, both Na and Cl are actively transported in the opposite direction, from lumen to plasma, while in the colon, only Na is actively transported with Cl moving passively.

Cooperstein and Hogben (9) have obtained evidence that in bullfrog large intestine *in vitro* an appreciable fraction of Cl movement takes place by "exchange diffusion." Such transport is presumably due to a non-energetic carrier system which cannot bring about any net transport against an electrochemical potential gradient (18). The data on Cl fluxes presented in Fig. 5 suggest that there is very little exchange diffusion involved in Cl movement in rat ileum *in vivo*. Consideration of the relationship between observed and calculated flux ratios for a true exchange diffusion system indicates that the following inequalities should hold (28). For $C_1\xi/C_2 > 1.0$, $\Phi_{12}/\Phi_{21} < C_1\xi/C_2$, and for $C_1\xi/C_2 < 1.0$, $\Phi_{12}/\Phi_{21} > C_1\xi/C_2$. In these expressions, $\xi = e^{zP(\psi_1 - \psi_2)/RT}$. As shown in Fig. 5 for Cl transport in rat colon, the observed values of $\Phi_{12}^{Cl}/\Phi_{21}^{Cl}$

are nearly equal to $C_1\xi/C_2$ at all points studied. Thus free diffusion appears to play the major role in Cl transport, and any exchange diffusion present makes a contribution too small to be detected by these experiments. In bull-frog large intestine *in vitro*, the free diffusion flux appears to be somewhat smaller than the exchange diffusion (9). Some of this difference may be due to species variation, but it is of interest to point out that free diffusion is considerably less in *in vitro* preparations of rat ileum than *in vivo* (29). This is presumably due to the fact that *in vitro* diffusion must take place across the whole intestinal wall rather than merely across the epithelial cell layer and into the plasma.

Model System for Na Transport

The present experiments have indicated that Na influx is dependent on Na concentration in the lumen (transconcentration). As discussed above, net Na transport across the mucosa could result in an increase in the amount of solute in the plasma while it is in contact with the mucosa. Thus, at higher NaCl concentrations in the lumen, the increased net absorption of Na might raise Na concentration in the plasma. If this effect occurs to a marked extent, Na flux from plasma to lumen should increase with increasing Na concentration as a result of a change in the Na concentration of the plasma in contact with the mucosa. However, such an explanation seems unlikely. As shown in Fig. 2 and Equation 1, water is transported at a rate nearly equal to the rate required to maintain absorbed solute at isotonic concentration. Consequently, the actual change in plasma Na concentration must be quite small. Further, no transconcentration effect has been observed for Cl movement although the rate of net Cl absorption is generally greater than that of Na.

A similar transconcentration effect has been observed in other biological systems, as for example, the Cl transport system of the frog stomach (30) and the Na transport system of the frog skin (31). In these cases, the effect has been interpreted in terms of a process of exchange diffusion *via* a carrier system involved in an active transport process. Such a mechanism may also be operative in the present case.

Na transport in the colon can be examined in terms of a model carrier transport system such as that shown in Fig. 7. The carrier substance is assumed to be confined to the membrane phase, and conserved in that phase. Therefore, in the steady state, the sum of the net fluxes of X and NaX from side 1 to 2 must be equal to the net fluxes of Y plus NaY from side 2 to 1. Net active transport of Na from side 1 to 2 will take place if $\Phi_{12}^{NaX} > \Phi_{21}^{NaY}$. This requires that the rate of formation of NaY at side 2 must be less than that of NaX at side 1. In general, such a system can actively transport Na from side 1 to 2 only if the carrier substance X is converted, at side 2, into

another substance *Y* which has a lower affinity for Na than does *X*. The metabolic energy necessary for active transport of Na from side 1 to 2 may be needed for conversion of *X* to *Y* on side 2, conversion of *Y* to *X* on side 1, or both. The model can explain the observed transconcentration effect if the membrane is more permeable to Na*X* and Na*Y* than to *X* and *Y*. Under these conditions, when Na concentration on side 1 is lowered, less Na*X* is formed at side 1 and the total flux of carrier from 1 to 2 will decrease. To maintain the steady state, the flux of Na*Y* from side 2 to 1 must also decrease. Thus, lowering Na concentration on side 1 will cause a decrease in Na flux from side 2 to side 1.

This model is a generalization of those proposed by Solomon (32) for K transport in red cells and by Heinz and Durbin (30) for Cl transport in frog gastric mucosa. A kinetic analysis of this system is given in the Appendix. A complete analysis (which may be carried out according to the methods de-

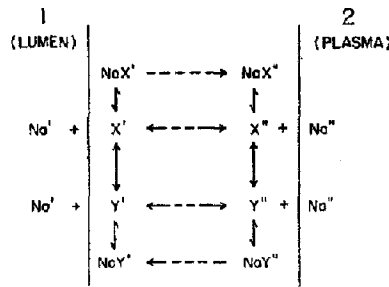


FIGURE 7. Model system for active Na transport in the colon.

scribed by Hearon (33, 34)) is very complex. Instead, the solution has been simplified by assuming that the concentrations of Na*Y* and *Y* on side 1 and Na*X* and *X* on side 2 are maintained very small. The necessary conditions for this simplification are given in the Appendix. These assumptions are primarily for mathematical simplicity; results analogous to those given below are obtained without these assumptions, but the complexity of the problem is greatly increased.

The final results of this analysis are given by Equations 3 and 4. These equations are obtained from the general solution by assuming that the only experimental variable is Na' (Na concentration in the lumen). The fluxes have been written as functions of this variable with the addition of terms describing free diffusion of Na.

$$\Phi_{12}^{Na} = \frac{K_1 Na'}{K_2 Na' + K_3} + K_2 Na' = \left[\frac{K_1}{K_2 Na' + K_3} + K_5 \right] Na' \quad (3)$$

$$\Phi_{21}^{Na} = \frac{K_1' Na' + K_4}{K_2 Na' + K_3} + K_5 Na'' \quad (4)$$

in which Φ_{12}^{Na} and Φ_{21}^{Na} are total fluxes of Na including both carrier transport and free diffusion, and the K 's (except K_5) are constants which describe the steady state behavior of the system as explained in the Appendix. K_5 is the permeability coefficient for Na diffusion.

The results of these calculations for the system shown schematically in Fig. 7 may be compared with the experimental results given in Fig. 3. Experimentally, both Φ_{12}^{Na} and Φ_{21}^{Na} are linear functions of Na' . Such results can be explained by the model system if $K_3 \gg K_2\text{Na}'$ for Na concentrations in the range 30 to 150 m.eq./liter. This condition implies that in this concentration range, the carrier system is far from saturation with Na; that is Φ_{12}^{NaX} and Φ_{21}^{NaY} (unidirectional Na fluxes not due to free diffusion of Na) should be linear functions of Na' .

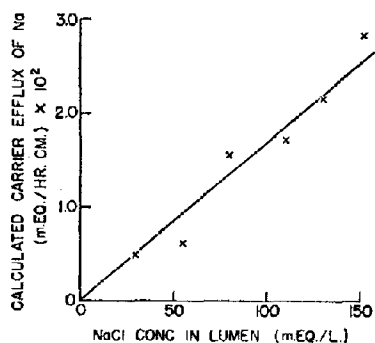


FIGURE 8. Calculated carrier-mediated Na efflux as a function of NaCl concentration in the lumen. The points represent the difference between total Na efflux and estimated simple diffusion flux. This estimation was based on the model system in Fig. 7 and was made by the method described in the text. The line has been drawn through the calculated points and has no theoretical significance.

Φ_{12}^{NaX} may be estimated, for the colon, by assuming that Na flux from plasma to lumen is entirely due to free diffusion when Na concentration in the lumen is zero. The diffusion permeability of the colon to Na can then be estimated from the plasma Na concentration and the Na influx extrapolated to zero Na concentration in the lumen. This calculation is subject to two restrictions. First, since no experiments were carried out at Na concentrations below 30 m.eq./liter, the extrapolation cannot be considered entirely accurate, but should give the proper order of magnitude. Second, the present model indicates (Equations 4 and A8) that Φ_{21}^{NaY} is not necessarily zero when Na' is zero, so that, even under this condition, Na flux from plasma to lumen may not be entirely due to free diffusion. If this is true, the calculated permeability coefficient will be too large. Subject to these conditions, the extrapolated permeability coefficient calculated in this way has a value of 3×10^{-5} cm.³/sec. cm. length of colon.¹ Using this extrapolated coefficient, Na efflux (Φ_{12}^{Na})

¹ This calculation was made using the assumption that the potential difference across the colon was zero. A potential oriented with the lumen relatively negative would result in an increased driving force on Na moving from plasma to lumen. With a given value of influx, any increased driving force would require a lower permeability coefficient. Since the lumen was found to be negative at all NaCl concentrations studied, the above value of the Na permeability coefficient probably represents a relatively high estimate.

can be separated into two terms, one due to diffusion and one due to carrier transport. Fig. 8 shows calculated carrier transport from lumen to plasma as a function of NaCl concentration in the lumen. This flux is a linear function of concentration indicating that there is no saturation of the carrier system in the range of concentrations studied. Thus, the present preliminary results are in agreement with a carrier transport model such as that illustrated in Fig. 7, but they do not constitute proof that such a system, or indeed, any carrier system is present in the colon.

Ion Permeability and Potential Difference

Since Cl crosses the mucosa mainly by simple diffusion, it is possible to calculate directly the permeability coefficient of the membrane for Cl. The diffusion permeability of the colon for Na and Cl can then be compared using the permeability coefficient for free diffusion of Na which has been calculated above. The average permeability coefficient of the membrane for Cl calculated from the observed fluxes, concentrations, and potentials is 6.0×10^{-5} cm.²/sec. cm. length. This coefficient is given by $(A/\Delta x)_{\text{Cl}} D_{\text{Cl}}$, in which A is the area available for diffusion, Δx is the thickness of the diffusion barrier, and D_{Cl} is the diffusion coefficient of Cl within the membrane. Further, an independent estimate of the value of $A/\Delta x$ for the colon can be obtained from the data on mannitol flux and the free diffusion coefficient of mannitol. This calculation yields a value of 2.0 cm./cm. length for $(A/\Delta x)_{\text{mannitol}}$. Since mannitol is uncharged, this $A/\Delta x$ may be considered to represent a measure of the geometric restriction to solute movement offered by the membrane, taking no account of any charge barrier which may exist. The restriction of Na and Cl movement resulting from geometric structure of the membrane should be roughly the same as for mannitol since the size of these solutes is of the same order of magnitude. Assuming that this value of $A/\Delta x$ applies to Na and Cl, apparent diffusion coefficients of these ions in the membrane may be calculated. The resulting values are 3.0×10^{-5} cm.²/sec. for Cl and 1.5×10^{-5} cm.²/sec. for Na. These data suggest that Cl diffuses more easily through rat colon than does Na. This conclusion rests on the validity of the extrapolation of Na influx to zero concentration; it is reinforced, however, by the observation that unidirectional Cl fluxes are generally greater than those of Na even though Na is actively transported.

These calculated diffusion coefficients of Na and Cl must include any effect of a charge barrier within the membrane on the rate of ion movement, and any appreciable difference between the two coefficients is indicative of the existence of such a barrier. Thus, the apparent difference between Na and Cl mobilities within the membrane could be explained by a positive charge barrier. In the ileum, the relationship between Na and Cl permeabilities is reversed; this tissue appears to be more permeable to Na than to Cl, and a

negative charge barrier in the membrane has been suggested (4). It is of further interest to note that the value of $A/\Delta x$ for mannitol obtained in the colon is only 55 per cent of the value obtained in rat ileum (4). Thus, the colon appears to be a tighter, less permeable membrane.

The relationship between potential difference across the colon and NaCl concentration in the lumen may be examined in light of these estimates of relative Na and Cl mobilities. Active Na transport appears to be the main source of the potential difference making the lumen negative relative to the plasma. As NaCl concentration in the lumen is reduced, the contribution of active Na transport to the potential should decrease, and the lumen might be expected to become less negative relative to the plasma. However, the NaCl concentration difference introduced across the membrane will give rise to a simple diffusion potential causing the lumen to become relatively negative due to the higher Cl mobility. Since these two changes are opposite in direction, they may partially balance one another in such a way that the total membrane potential shows little variation with changes in NaCl concentration.

In conclusion, there are several marked differences between rat ileum and colon with respect to Na and Cl transport. Na is actively transported by the colon while Cl movement is passive. Consequently, an electrical potential difference may be maintained across the mucosa when identical solutions are placed on both sides. Such a potential is not observed in the ileum since both Na and Cl are actively transported in the same direction. The colon appears to be generally less permeable than the ileum, and appears to have a charge barrier which is opposite in sign to that previously suggested for the ileum.

The authors would like to thank Dr. A. K. Solomon for suggesting this problem and for his many helpful discussions during the course of the work.

APPENDIX

The model system shown in Fig. 7 may be described, in the steady state, by the following set of equations.

$$\begin{aligned}
 J &= w_1 X' - w_{-1} \text{Na}X' + p_2(X' - X'') \\
 &= p_1(\text{Na}X' - \text{Na}X'') + p_2(X' - X'') \\
 &= w_2 \text{Na}X'' - w_{-2} X'' + p_2(X' - X'') \\
 &= w_3 X'' - w_{-3} Y'' \\
 &= w_4 Y'' - w_{-4} \text{Na}Y'' + p_3(Y'' - Y') \\
 &= p_3(Y'' - Y') + p_4(\text{Na}Y'' - \text{Na}Y') \\
 &= w_5 \text{Na}Y' - w_{-5} Y' + p_3(Y'' - Y') \\
 &= w_6 Y' - w_{-6} X'
 \end{aligned} \tag{A1}$$

in which $w_1 = k_1 \text{Na}'$, $w_{-1} = k_{-1}$, etc. Thus the terms w_i contain rate constants and, where necessary, the concentration of reactants other than carrier species. \bar{J} is the steady reaction rate, and the p_i are permeability coefficients. The symbols X , $\text{Na}X$, etc. refer to concentrations and the superscripts prime and double prime to sides 1 and 2 of the membrane respectively. Since the carrier is assumed to be confined to the membrane phase, the following conservation equation for carrier substance also applies.

$$X_t = X' + X'' + \text{Na}X' + \text{Na}X'' + Y' + Y'' + \text{Na}Y' + \text{Na}Y'' \tag{A2}$$

in which X_t is total concentration of carrier in all forms.

The set of equations A1 with Equation A2 may then be solved simultaneously for \bar{J} . The complete solution of this set is unwieldy but the problem is considerably simplified by assuming that reactions 3 and 6 are fast and irreversible ($w_{-3} = w_{-6} = 0$) and that reactions 2 and 5 are fast (w_2 and w_5 are large; $\text{Na}X''$ and $\text{Na}Y'$ are then very small). These assumptions are primarily for mathematical simplicity since similar, but more complex results may be obtained without them. Under these conditions, \bar{J} is given by equation A3.

$$\bar{J} = \frac{X_t \alpha \beta}{\alpha(w_4 + w_{-4} + p_4) + \beta(w_1 + w_{-1} + p_1)} \tag{A3}$$

in which

$$\alpha = w_1 p_1 + w_{-1} p_2 + p_1 p_2$$

$$\beta = w_4 p_4 + w_{-4} p_3 + p_3 p_4$$

The steady state concentrations $\text{Na}X'$ and $\text{Na}Y''$ are given by Equations A4 and A5.

$$\text{Na}X' = \frac{w_1 \tilde{f}}{\alpha} = \frac{w_1 \beta X_t}{\alpha(w_4 + w_{-4} + p_4) + \beta(w_1 + w_{-1} + p_1)} \quad (\text{A4})$$

$$\text{Na}Y'' = \frac{w_4 \tilde{f}}{\beta} = \frac{w_4 \alpha X_t}{\alpha(w_4 + w_{-4} + p_4) + \beta(w_1 + w_{-1} + p_1)} \quad (\text{A5})$$

Assuming that all other terms are constant, the relationship between fluxes and Na' may be examined; (Na' is analogous to Na concentration in the lumen of the colon). In the above equations, the only terms containing Na' are those containing w_1 . The carrier-mediated Na fluxes in the two directions across the membrane are then:

$$\Phi_{12}^{\text{Na}X} = p_1 \text{Na}X' = \frac{K_1 \text{Na}'}{K_2 \text{Na}' + K_3} \quad (\text{A6})$$

$$\Phi_{21}^{\text{Na}Y} = p_4 \text{Na}Y'' = \frac{K_1' \text{Na}' + K_4}{K_2 \text{Na}' + K_3} \quad (\text{A7})$$

in which

$$K_1 = k_1 p_1 X_t \beta$$

$$K_1' = k_1 p_1 p_4 w_4 X_t$$

$$K_2 = k_1 (\beta + w_4 p_1 + w_{-4} p_1 + p_1 p_4)$$

$$K_3 = (w_{-1} + p_1) \beta + (w_4 + w_{-4} + p_1) (p_1 p_2 + w_{-1} p_2)$$

$$K_4 = p_4 w_4 X_t (p_1 p_2 + w_{-1} p_2)$$

Thus, the carrier fluxes in both directions are dependent on Na concentration on side 1. The net Na flux in this system is equal to $(\Phi_{12}^{\text{Na}X} - \Phi_{21}^{\text{Na}Y})$. Examination of Equations A6 and A7 shows that net Na transport from side 1 to 2 will take place if $w_1 w_{-4} > w_{-1} w_4$; that is, if X has a higher affinity for Na than does Y. The total unidirectional Na fluxes across such a membrane, measured by means of tracers, would include both carrier transport and free diffusion of Na. These fluxes would be given by the following equations.

$$\Phi_{12}^{\text{Na}} = \frac{K_1 \text{Na}'}{K_2 \text{Na}' + K_3} + K_5 \text{Na}' \quad (\text{A8})$$

$$\Phi_{21}^{\text{Na}} = \frac{K_1' \text{Na}' + K_4}{K_2 \text{Na}' + K_3} + K_5 \text{Na}'' \quad (\text{A9})$$

in which Φ_{12}^{Na} and Φ_{21}^{Na} are total fluxes of Na due to both diffusion and carrier transport, and K_5 is the permeability coefficient of the membrane for Na diffusion.

It is necessary to point out that this is an entirely general model system having sufficient adjustable constants to fit many types of experimental results. A complete test of the applicability of such a model to any actual transport system requires estimation of at least some individual w 's. The kinetic analysis of the system has been presented here since a knowledge of these results is essential for any further experimental attempt to test the model.

BIBLIOGRAPHY

1. D'AGOSTINO, A., LEADBETTER, W. F., and SCHWARTZ, W. B., *J. Clin. Inv.*, 1953, **32**, 444.
2. PARSONS, D. S., *Quart. J. Exp. Physiol.*, 1956, **41**, 410.
3. BURNS, H. S., and VISSCHER, M. B., *Am. J. Physiol.*, 1934, **110**, 490.
4. CURRAN, P. F., and SOLOMON, A. K., *J. Gen. Physiol.*, 1957, **41**, 143.
5. CURRAN, P. F., SCHWARTZ, G. F., and SOLOMON, A. K., Abstracts, The Biophysical Society, Cambridge, Massachusetts, 1958.
6. COOPERSTEIN, I. L., and BROCKMAN, S. K., *J. Clin. Inv.*, 1959, **38**, 435.
7. COOPERSTEIN, I. L., private communication.
8. USSING, H. H., and ANDERSEN, B., *Proc. 3rd Internat. Congr. Biochem.*, 1955, p. 435.
9. COOPERSTEIN, I. L., and HOGBEN, C. A. M., *J. Gen. Physiol.*, 1959, **42**, 461.
10. SOLOMON, A. K., and CATON, D. C., *Anal. Chem.*, 1955, **27**, 1949.
11. SCHALES, O., and SCHALES, S. S., *J. Biol. Chem.*, 1941, **140**, 879.
12. BRUN, C., *Nord. Med.*, 1949, **42**, 1774.
13. MACFADYEN, D. A., *J. Biol. Chem.*, 1945, **158**, 107.
14. KARNOVSKY, M. L., and BRUMM, A. F., *J. Biol. Chem.*, 1955, **316**, 689.
15. ROBINSON, C. V., *Science*, 1950, **112**, 198.
16. DURBIN, R. P., CURRAN, P. F., and SOLOMON, A. K., *Adv. Biol. and Med. Physics*, 1958, **6**, 1.
17. ROSENBERG, T., *Acta Chem. Scand.*, 1948, **2**, 14.
18. USSING, H. H., *Acta Physiol. Scand.*, 1949, **19**, 43.
19. KOEFOED-JOHNSON, V., and USSING, H. H., *Acta Physiol. Scand.*, 1953, **28**, 60.
20. HODGKIN, A. L., and KEYNES, R. D., *J. Physiol.*, 1955, **128**, 61.
21. VISSCHER, M. B., ROEPKE, R. R., and LIFSON, N., *Am. J. Physiol.*, 1945, **144**, 457.
22. VISSCHER, M. B., and ROEPKE, R. R., *Am. J. Physiol.*, 1945, **144**, 468.
23. MCHARDY, G. J. R., and PARSONS, D. S., *Quart. J. Exp. Physiol.*, 1957, **42**, 33.
24. GOLDSCHMIDT, A., and DAYTON, A. B., *Am. J. Physiol.*, 1919, **48**, 450.
25. COLE, D. F., and MEREDITH, J. F., *Bull. Math. Biophysics*, 1957, **19**, 23.
26. ATKINSON, R. M., PARSONS, B. J., and SMYTH, D. H., *J. Physiol.*, 1957, **135**, 581.
27. HOGBEN, C. A. M., *Am. J. Physiol.*, 1955, **180**, 641.
28. CURRAN, P. F., Thesis, Harvard University, 1957.
29. CURRAN, P. F., and SOLOMON, A. K., private communication.
30. HEINZ, E., and DURBIN, R. P., *J. Gen. Physiol.*, 1957, **41**, 101.
31. KIRSCHNER, L. B., *J. Cell. and Comp. Physiol.*, 1955, **45**, 61.
32. SOLOMON, A. K., *J. Gen. Physiol.*, 1952, **36**, 57.
33. HEARON, J. Z., *Bull. Math. Biophysics*, 1949, **11**, 29.
34. HEARON, J. Z., *Bull. Math. Biophysics*, 1949, **11**, 83.