Na, Cl, and Water Transport by Rat Ileum *in Vitro*

PETER F. CURRAN

From the Biophysical Laboratory, Harvard Medical School, Boston, Massachusetts, and the Institute for Biological Chemistry, the University of Copenhagen, Copenhagen, Denmark. Dr. Curran's present address is the Biophysical Laboratory, Harvard Medical School, Boston.

ABSTRACT Interrelationships between metabolism, NaCl transport, and water transport have been studied in an *in vitro* preparation of rat ileum. When glucose is present in the mucosal solution, Na and Cl both appear to be actively transported from mucosa to serosa while water absorption is passive and dependent on net solute transport. Removal of glucose from the mucosal solution or treatment with dinitrophenol, monoiodoacetate, or anoxia inhibits active salt transport and as a result, water absorption is also inhibited. The dependence of water absorption on metabolism can be explained as a secondary effect due to its dependence on active salt transport. The relationship between salt and water transport has been discussed in terms of a model system.

In experiments on rat ileum *in vivo*, Curran and Solomon (1) have obtained evidence indicating that water transport is a passive process which takes place as a result of active solute transport. On the other hand, Fisher (2) and Smyth and Taylor (3) have found that water absorption by *in vitro* preparations of rat small intestine is inhibited by absence of glucose in the mucosal solution and by a variety of metabolic poisons. These results have led them to suggest that water transport may be, at least in part, due to some active process. However, Curran and Solomon have proposed that this dependence of water transport on metabolism is a reflection of its dependence on active NaCl transport. The present experiments were carried out in an attempt to clarify the interrelationships between metabolism, NaCl transport, and water transport, and to examine further the effects of glucose and metabolic poisons on ion transport by rat ileum *in vitro*.

METHODS

The perfusion technique used by Curran and Solomon (1) has been modified for use with *in vitro* preparations of intestine. In one type of experiment, the abdomen of an

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anesthetized rat was opened, a segment of distal ileum about 10 cm. long selected, and lucite cannulae inserted into each end. The segment was then cut free from the animal and the cannulae were inserted into the ends of a rectangular lucite chamber. The tube of intestine was immediately filled with oxygenated perfusing fluid. A $NaCl$–$NaHCO_3$ buffer (composition given in Table I, reference 4) was placed in the chamber in contact with the serosal side of the intestine and the lumen was perfused with test solution using the method previously described (1). The perfusing fluids were either NaCl solutions or mixtures of NaCl and mannitol to which small amounts of human hemoglobin, $Na^{24}Cl$, and $NaCl^{36}$ were added. As in previous experiments (1), hemoglobin served as a reference substance for determination of net water transport. Glucose was present at a concentration of 14 mM/liter. In some experiments, the glucose in the perfusing fluid was replaced by an equivalent amount of mannitol. The total osmolar concentrations of the solutions on the two sides of the membrane were approximately equal. In these experiments, unidirectional and net fluxes of Na and Cl and the net flux of water were measured.

A second type of experiment was used to study the effects of metabolic poisons on unidirectional ion fluxes. In order to study mucosal to serosal fluxes, a segment of ileum was removed from an animal and everted following the method of Wilson and Wiseman (5). Cannulae were inserted and the preparation mounted in the lucite chamber. Solution containing Na^{24} or Cl^{36} was placed in the chamber in contact with the mucosal surface and fluid was pumped slowly through the lumen of the tube (serosal side) using a peristaltic action pump. Fluid flowing out of the intestine was collected for fixed intervals of time, usually 5 or 10 minutes, and the total amount of radioactivity in the samples was determined. Collections were usually begun one-half hour after mounting the segment. In each segment, normal flux from mucosa to serosa was first determined for one-half hour before the poison to be tested was added to the mucosal solution. In some experiments, serosal to mucosal fluxes were measured using the same method. In this case, the intestine was not everted.

In experiments using this second method, the solution bathing the serosal surface was a $NaCl$–$NaHCO_3$ buffer while the mucosal fluid usually contained 150 mM/liter NaCl and 14 mM/liter glucose. In all experiments, the mucosal solution was continually bubbled with 100 per cent O_2 and the serosal solution with 95 per cent O_2–5 per cent CO_2. To test the effect of anoxia, the mucosal solution was bubbled with 100 per cent N_2. All experiments were carried out with the chamber in a constant temperature air bath maintained at 37°C.

In the first type of experiment, Na and Cl fluxes were calculated using the equations previously presented (1). In the second type of experiment, fluxes were calculated from the following equations.

$$\phi_{ms} = \frac{60\,S}{lt\,\bar{\bar{m}}}; \qquad \phi_{sm} = \frac{60\,M}{lt\,\bar{s}}$$

in which ϕ_{ms} is flux from mucosa to serosa and ϕ_{sm} is flux from serosa to mucosa. S and M represent total amount of radioactivity appearing on the serosal and mucosal sides respectively in the time t; $\bar{s}$ and $\bar{m}$ are specific activities of Na^{24} or Cl^{36} in the serosal and mucosal solutions and l is the length of the intestinal segment.

Hemoglobin concentration was determined by measuring optical density at 412 mμ on a Beckman model B spectrophotometer. Na concentration was determined on the flame photometer described by Solomon and Caton (6) and Cl concentration by the method of Schales and Schales (7). Na^{24} gamma rays were determined in a well type scintillation counter and Cl^{36} beta rays in a windowless proportional flow counter (8). In experiments carried out at Copenhagen, all counting was done with an end-window Geiger tube. Electrical potential difference between mucosal and serosal solutions was measured using calomel electrodes connected to the solutions by NaCl–agar bridges. A Keithley model 200B direct current electrometer was used as a detector in experiments at Harvard and a radiometer model PHM3 meter in experiments at Copenhagen.

RESULTS

Experiments with glucose present in the mucosal solution yielded results essentially similar to those obtained earlier *in vivo* (1). As shown in Fig. 1, net water transport from isotonic solutions was dependent on the rate of net Na transport, and no significant water absorption from these solutions was found in the absence of net Na transport. (Mannitol has not been included in net solute transport; measurement of mannitol transport in two experiments indicated that it was negligible relative to Na transport.) As previously discussed (1, 9), this observation indicates that water transport is a passive process depending only on gradients of water activity.

In Fig. 2, the observed ratios of unidirectional Na and Cl fluxes found in this series of experiments are plotted against the ratios expected if the ions cross the membrane independently and under the influence of concentration and electrical potential gradients only (10, 11). With glucose present in the mucosal solution, the points for both Na and Cl lie well above the line representing equality of observed and expected values indicating that Na and Cl movements cannot be explained entirely by concentration and electrical forces. In the absence of glucose, the observed flux ratios become nearly equal to those expected for passive transfer.

In four experiments with glucose (three for Na and one for Cl), water flow was nearly zero or net water transfer from serosa to mucosa occurred. A comparison of observed and expected flux ratios under these conditions is useful in considering the possible effect of solvent drag (11) on the results presented in Fig. 2. The results of these experiments are given in Table I. In each case, the observed flux ratio is greater than that expected.

Table II summarizes the results of measurements of Na and Cl fluxes and net water flux from 150 mM/liter NaCl solutions in the presence and absence of glucose. The results of earlier *in vivo* experiments are included for comparison. When glucose in the mucosal solution is replaced by mannitol, there is no significant net transport of Na, Cl, or water and the mucosal to serosal Na and Cl fluxes are markedly reduced.

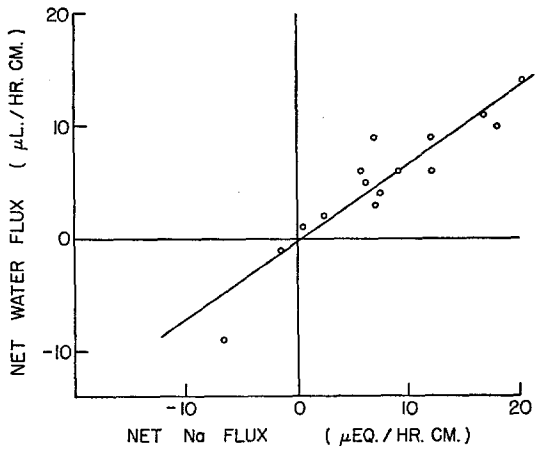

FIGURE 1. Relationship between net water flux and net Na flux with glucose present in the mucosal solution. The solutions had varying NaCl concentrations but were kept isotonic by addition of mannitol. The line has been determined by the method of least squares.

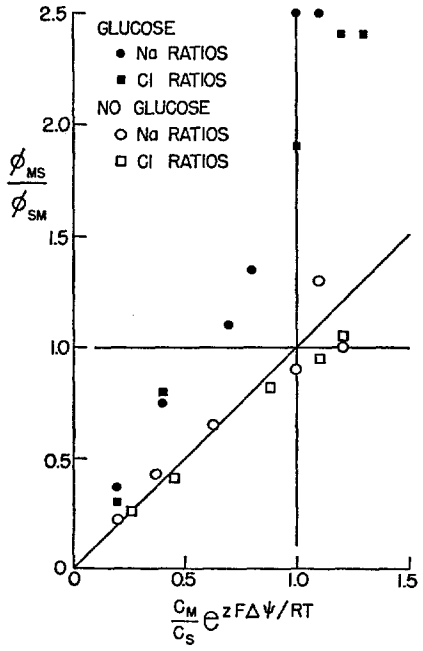

FIGURE 2. Relationship between observed and expected Na and Cl flux ratios. The line represents equality of observed and expected values. ϕ_{ms} is mucosal to serosal flux and ϕ_{sm} is serosal to mucosal flux. C_m and C_s are, respectively, mucosal and serosal ion concentrations; $\Delta\psi$ is electrical potential difference, z, ion charge, F, the Faraday, R, the gas constant, and T, the absolute temperature.

The second experimental method described above has been used to study the effects of 2,4-dinitrophenol (DNP), sodium monoiodoacetate (IAA) and anoxia on Na and Cl fluxes. Such agents are known to inhibit water transport (3), but their effects on ion fluxes have not been extensively studied. The results of a control experiment for Na flux in which inhibitor was not used are shown in Fig. 3. The Na flux remained relatively constant over a 3 hour period. The effect on Na flux of stopping glucose absorption without the addition of a metabolic inhibitor is illustrated in Fig. 3. The intestine was first perfused for 1 hour with glucose present; the normal mucosal solution was then replaced by one containing no glucose. Na flux remained constant for approximately 40 minutes and then declined gradually.

TABLE I

Na AND Cl FLUX RATIOS

Net water flux*	Ion	Expected flux ratio	Observed flux ratio
µl./hr. cm.			
10	Na	0.69	1.10
−10	Na	0.39	0.75
−90	Na	0.20	0.37
−90	Cl	0.20	0.30

* A negative flux indicates net movement from serosa to mucosa.

TABLE II

EFFECT OF GLUCOSE ON ION AND WATER FLUXES

	Na flux*			Cl flux*			Net water flux
	ϕ_{ms}	ϕ_{sm}	ϕ_n	ϕ_{ms}	ϕ_{sm}	ϕ_n	
	µeq./hr. cm.			*µeq./hr. cm.*			*µl./hr. cm.*
In vivo	54.0	38.0	16.0	31.0	18.0	13.0	115
In vitro (glucose)	20.9	9.3	11.9	22.4	10.2	12.2	80
In vitro (no glucose)	6.5	6.4	0.1	7.6	7.9	−0.3	1
p‡	<0.01	>0.05	<0.01	<0.01	>0.05	<0.01	<0.01

* ϕ_{ms}, ϕ_{sm}, and ϕ_n are mucosal to serosal flux, serosal to mucosal flux, and net flux respectively.
‡ p refers to the significance of the difference between *in vitro* results with and without glucose.

The effects of IAA on mucosal to serosal Na and Cl fluxes are illustrated in Fig. 4. DNP and anoxia caused similar depression in flux but both were found to act more rapidly than IAA. The mean time required to reach a minimum flux level after addition of poison was 30 minutes for IAA, 25 minutes for anoxia, and 15 minutes for DNP. The results of this series of experiments are summarized in Table III. The poisons used all reduced mucosal to serosal Na and Cl fluxes appreciably. Net ion transport in the presence of poisons has

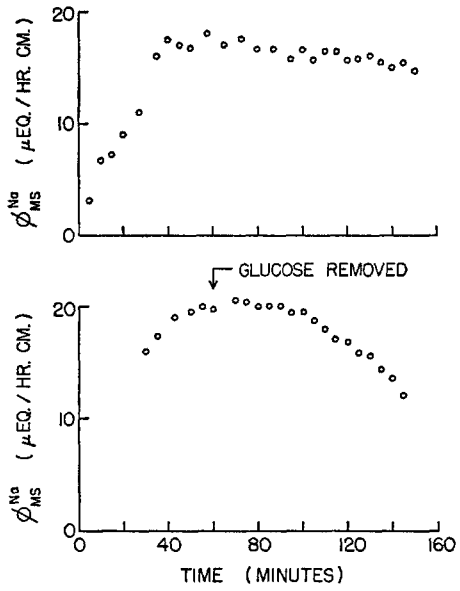

FIGURE 3. Mucosal to serosal Na flux as a function of time. Top, control experiment with glucose present. The initial rising phase represents time necessary to establish a steady tracter flux. Bottom, effect of stopping glucose absorption. At the time indicated by the arrow, the normal mucosal solution was replaced by one containing no glucose.

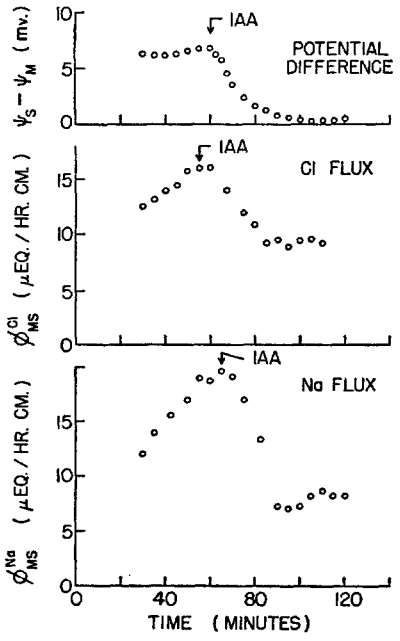

FIGURE 4. Effect of IAA (1×10^{-3} M) on electrical potential difference and mucosal to serosal Na and Cl fluxes. Glucose present.

been calculated by assuming that the opposing flux (from serosa to mucosa) is not altered by poisons. This assumption appears valid since experiments have shown that DNP and IAA do not affect this opposing Na flux appreciably. IAA increased serosal to mucosal flux by 15 per cent while DNP decreased it by 10 per cent, but the changes were not statistically significant.[1] Thus, it is reasonable to conclude that in the presence of these metabolic poisons there is little net transport of Na or Cl.

TABLE III
EFFECT OF METABOLIC POISONS ON ION FLUX

	Control	DNP (5×10^{-4} M)	IAA (10^{-3} M)	Anoxia
Na flux* $\mu eq./hr. cm.$				
ϕ_{ms}	21.2 ± 0.6‡	8.8 ± 0.8	9.3 ± 0.8	10.4 ± 0.4
ϕ_{sm}	8.7 ± 0.3			
ϕ_n	12.5 ± 0.7	0.1 ± 0.9	0.5 ± 0.9	1.7 ± 0.5
Cl flux $\mu eq./hr. cm.$				
ϕ_{ms}	17.4 ± 0.8	9.8 ± 0.6	10.7 ± 0.5	9.5 ± 0.4
ϕ_{sm}	9.2 ± 0.4			
ϕ_n	8.2 ± 0.9	0.6 ± 0.7	1.5 ± 0.7	0.3 ± 0.6

* ϕ_{ms}, ϕ_{sm}, and ϕ_n are mucosal to serosal flux, serosal to mucosal flux, and net flux respectively.
‡ Errors are given as standard error of the mean.

DISCUSSION

The results of the present experiments on *in vitro* preparations of rat intestine compare well with those of earlier *in vivo* experiments (1). As shown in Table II, the net fluxes of Na, Cl, and water found with glucose present in the mucosal solution are relatively close to those observed *in vivo*. The unidirectional Na and Cl fluxes *in vitro* are considerably lower than those found *in vivo*. This is most likely due to the fact that *in vitro* transport must take place across the muscle layer instead of simply across the epithelial cells and into the plasma. This added diffusion barrier would be expected to reduce unidirectional fluxes.

Active Ion Transport

The results presented in Fig. 2 indicate that when glucose is present in the mucosal solution Na and Cl transport is not due entirely to simple diffusion. A

[1] These values are not included in Table III since the experiments were carried out on a strain of rats different from those used in other Na experiments.

difference between observed and expected flux ratios may be due to active transport, but it could also be the result of solvent drag, exchange diffusion, or single file diffusion. However, neither of the last two effects can bring about net transport and they can be ruled out by the observation that net transport of both Na and Cl takes place in the absence of an electrochemical potential gradient. This is shown by the fact that, in Fig. 2, $\phi_{ms}/\phi_{sm} > 1.0$ when $C_m\xi/C_s = 1.0$ ($\xi = e^{zF\Delta\psi/RT}$). It is further confirmed in the case of Na by the control fluxes found in the series of experiments reported in Table III. In these experiments, Na concentration was the same on both sides of the intestine and the electrical potential difference, although small (2 to 6 mv.), was oriented with the serosa positive relative to the mucosa. Thus, the observed net Na transport took place against an electrochemical potential gradient.

As shown by Koefoed-Johnson and Ussing (reference 11, Equations 12 and 13), the effect of solvent drag on flux ratios depends on the magnitude and direction of net water flow. It may be described by an equation of the following form.

$$\frac{\phi_{ms}}{\phi_{sm}} = \left[\frac{C_m\xi}{C_s}\right] e^{b\phi_n^w}$$

in which ϕ_n^w is net water flux and b is a coefficient depending on the nature of the solute and the structure of the membrane. Thus, if a difference between ϕ_{ms}/ϕ_{sm} and $C_m\xi/C_s$ is entirely due to solvent drag, ϕ_{ms}/ϕ_{sm} should be equal to $C_m\xi/C_s$ when ϕ_n^w is near zero and should be less than $C_m\xi/C_s$ when ϕ_n^w is negative (net water movement from serosa to mucosa in the present notation). The data presented in Table I show that ϕ_{ms}/ϕ_{sm} remains greater than $C_m\xi/C_s$ under these conditions indicating that solvent drag does not play a dominant role in Na and Cl transport.

Some additional caution is necessary in interpreting the results for Cl. In studies of net Cl transport by everted sacs of rat ileum, Curran and Thale (12) have recently observed that the intestine gains Cl during absorption *in vitro*. They found a relatively large disappearance of Cl from the mucosal solution but only a small net transfer into the serosal solution. This may lead to error in fluxes measured with tracers since the intestine will not be in a steady state with respect to Cl. The magnitude of such an effect cannot be evaluated with the present data. Subject to this reservation, the results presented in Fig. 2 indicate that both Na and Cl are actively transported from mucosa to serosa when glucose is available.

Under conditions which inhibit metabolism, active ion transport no longer takes place. As shown in Fig. 2, the flux ratios for Na and Cl are consistent with passive transfer when glucose is not present in the mucosal solution. After treatment with DNP, IAA, or anoxia, the electrical potential difference across the intestine falls to zero as shown in Fig. 4. Under these conditions, the flux

ratios expected for passive diffusion are 1.0 for Na and 1.25 for Cl (Cl concentration in the mucosal solution was higher than in the serosal solution). The ratios observed, obtained by averaging the results for all three poisons as given in Table III, are 1.1 for both Na and Cl. This figure is not exact, but it indicates that active transport has been greatly reduced by treatment with these agents.

Since active ion transport does not take place in the absence of glucose, it is possible that metabolic poisons inhibit active transport as a result of inhibition of glucose absorption. However, the results in Figs. 3 and 4 indicate that the rate of change in flux is much more rapid after addition of metabolic poisons than after removal of glucose from the bathing solution. Thus, DNP, IAA, and anoxia presumably influence ion fluxes directly as a result of their well known effects on the metabolic processes which must supply the energy necessary for active ion transport. The slow change in flux following glucose removal can be explained by the observation of Fisher and Parsons (13) that there is an accumulation of glucose in the wall of rat intestine *in vitro* during a period of glucose absorption. This accumulated glucose can apparently support active transport for some time after glucose has been removed from the bathing solution.

Water Transport

The data presented in Fig. 1 show that when isotonic solutions are placed on both sides of the intestine *in vitro* there is no net water transport unless there is a net solute movement. This observation indicates that water transport is a passive process since it shows that net water movement cannot take place in the absence of a water activity gradient. With isotonic solutions on both sides, a gradient of water activity can be supplied by net solute transport. Similar results have been obtained for rat ileum and colon *in vivo* and the conclusion has been discussed fully elsewhere (1, 9).

Under the conditions of the present experiments, net solute (NaCl) transport is an active, energy-requiring process which does not take place in the absence of glucose. Since Fig. 1 shows that net Na absorption is necessary for water absorption, no net water transport can be expected when glucose is not added to the mucosal solution. Further, DNP and IAA inhibit water absorption *in vitro* (3). The data presented in Table III indicate that these agents also inhibit active net Na transport and a reduction in water transport can, therefore, be expected. The dependence of water absorption on metabolism is secondary to its dependence on active solute transport and does not necessarily imply that water is actively transported.

A possible mechanism by which active NaCl transport brings about net water movement in intestine *in vivo* has been discussed by Curran and

Schwartz (9). The essential feature of this mechanism is the requirement that the active solute transport cause a transient rise in solute concentration of the plasma in contact with the absorbing cells. The osmotic gradient thus created would cause water movement and explain the observation that solute absorption provides the major driving force for water absorption. A similar mechanism may also be considered for water transport *in vitro* although some modifications are essential in order to take into account important experimental observations.

Parsons and Wingate (14) have recently observed in rat intestine *in vitro* that net water movement may take place from mucosal solution to serosal solution even when the solute concentration on the mucosal side is greater than that on the serosal side. That is, they have found net transport of water

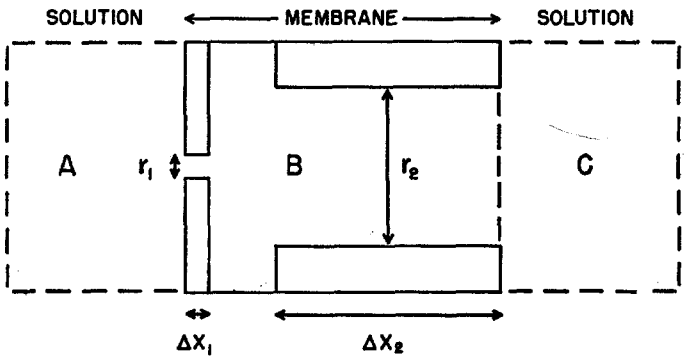

FIGURE 5. Schematic model system for water transport. *A* and *C* represent external solution. *r* and Δx represent pore radius and membrane thickness respectively.

from a lower to a higher chemical potential. This appears to contradict the conclusion that water transport is passive, and the observation must be explained by any theory of water transport by intestine. Further, hydrostatic pressure applied to the mucosal surface *in vitro* has only a slight effect on water transport (2, 3) but a low hydrostatic pressure applied to the serosal surface of hamster intestine will stop net water transfer (15). Raising solute concentration in the mucosal solution to a relatively high level will stop net water absorption (3, 14). Finally, solution will emerge from the serosal surface of the intestine even if there is no solution bathing this surface (3). This last observation suggests that the driving force for water transport is not entirely an osmotic pressure gradient but that it could be a hydrostatic pressure gradient generated within the tissue itself.

A working hypothesis incorporating these observations can be developed to explain water transport as a passive process linked to active solute transport. The hypothesis will be discussed in terms of the schematic model illustrated

in Fig. 5 in which two solutions of equal solute concentration are separated by a complex membrane which must be considered as a separate compartment. This membrane is made up of two porous structures in series, a thin membrane with small pores and a much thicker membrane with large pores. It will be assumed that solute is actively transported across the thin membrane from region A to B. In the intestine *in vitro*, the thin membrane might be represented by one of the membranes of the epithelial cells while the thick membrane might be the submucosal and muscle layers.

Active solute transport will cause a relatively high solute concentration in region B, and the resulting osmotic pressure gradient will cause water to move across the membrane from A to B. This gradient will cause little water movement from C to B because of the large pores in this region. The theoretical considerations of Staverman (16) have shown that for membranes permeable to solute, the effective osmotic pressure will be less than the theoretical pressure by a factor which depends on the relative sizes of the pores and the solute molecules. Durbin (17) has shown that for small solutes and relatively large pores the effective osmotic pressure may be quite close to zero. Thus, the osmotic pressure gradient between B and C will be small if the pores are sufficiently large. Entrance of water into region B from A will, however, cause an increase in hydrostatic pressure unless the compartment can expand freely. This pressure would then drive water through the large pores from B to C, but would have little effect on water flow from B to A because of the much smaller radius of the pores between these regions. The net result would be movement of water from A to C without requiring active water transport.

Such a model is capable of explaining, at least in theory, many observations on water transport *in vitro*. Thus, a small hydrostatic pressure at C could prevent water flow while a pressure applied at A would have a much smaller effect because of the small pore radius. The observations of Parsons and Wingate (14) could be explained by such a system. Water could be transported from A to C even if the solute concentration in A is greater than that in C as long as active solute transport takes place at a rate sufficiently rapid to maintain concentration in B greater than in A. This hypothesis thus serves to illustrate that the available data on water movement in intestine *in vitro* can be interpreted in terms of passive water transport coupled with active solute transport.

In conclusion, the present experiments have shown that the dependence of intestinal water transport on metabolism is of a secondary nature. Under normal conditions, both Na and Cl are actively transported from mucosa to serosa. Water transport is passive and is determined by the active solute transport which is dependent on metabolism. The inhibition of metabolism results in a decrease in active solute transport which, in turn, causes a decrease in water absorption. The relationship between solute and water transport can

be explained in terms of hydrostatic and osmotic pressure gradients within the tissue which are determined by active solute transport.

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