Effects of pressure on water and solute transport by dog intestinal mucosa in vitro

A. A. HAKIM AND NATHAN LIFSON

Department of Physiology, University of Minnesota, Minneapolis, Minnesota 55455

HAKIM, A. A., AND NATHAN LIFSON. Effects of pressure on water and solute transport by dog intestinal mucosa in vitro. Am. J. Physiol. 216(2): 276-284. 1969.—This paper describes in some detail the relationship between hydrostatic pressure and fluid transport found for the dog intestinal mucosa in vitro. With glucose-containing Krebs-bicarbonate-Ringer solution as the bathing fluids, the rate of fluid absorption was not affected by excess mucosal pressures up to 20 cm H₂O. By contrast, excess serosal pressures of about 4 cm H₂O reduced fluid transport to zero and higher serosal pressures reversed absorption to secretion. Excess serosal pressure very probably increases the hydraulic permeability coefficient by widening existing, or opening new channels. The kinetics of solute transport during the secretory flow is consistent with this view. Consideration is given to the possible relevance of the asymmetry of the pressure sensitivity to in vivo intestinal function. Misconceptions concerning the interpretation of unidirectional fluxes of water during intestinal absorption or secretion are discussed.

METHODS

Sheets of dog small intestinal mucosa consisting only of epithelium and lamina propria were mounted for study as previously described (11). In essence, the membranes were tied on to one end of a cylindrical glass tube and the membrane-covered end of the tube was immersed in a large volume (usually 200 ml) of bathing fluid. A much smaller volume of fluid was placed in the tube. Which of the two fluids bathed the epithelial ("mucosal") surface and which the trans-epithelial ("serosal") surface of the sheet of mucosa depended on whether the mucosal surface faced the inside or outside of the tube. Both orientations were employed. Membranes obtained from the oral half of the jejunum ileum will be designated as jejunal; those from the aboral half, as ileal. Both the mucosal and serosal bathing fluids were Krebs-bicarbonate-Ringer solution (25) containing one-half the described calcium content and sufficient glucose not to limit water absorption (usually 200 or 500 mg/100 ml). In several instances other solutes were added to the Ringer solution (see RESULTS). Excess pressure, either serosal or mucosal, was provided by suitable adjustment of the relative heights of the surfaces of the two bathing fluids. For the higher pressures employed, the dimensions of the membrane-bearing tube or outer chamber were modified as required. Sometimes suction on one side was used, but there was no obvious difference in the results when the

SINCE LUMINAL HYDROSTATIC pressure is an obvious possibility as a driving force for intestinal absorption, its effects on water transfer have been investigated a number of times in the past. Conclusions vary greatly. At one extreme is the view that the chief driving force for isotonic water movement is indeed the imbalance between a) luminal hydrostatic pressure plus villus tissue fluid colloid osmotic pressure, and b) villus tissue fluid hydrostatic pressure (28, 30). Certainly isotonic water absorption cannot all be explained in this way, and the most widely accepted judgment is that it is secondary to active solute transport (20).

At the other extreme is the view that the luminal distension pressure has negligible effects on the essential mechanism except via secondary factors, such as effective mucosal area, blood flow, or, in in vitro preparations, the oxygenation of the epithelium.

We have very briefly reported observations on the relationship between hydrostatic pressure and fluid transport for a recently developed in vitro preparation of canine intestinal mucosa (11, 12). The physiological relevance of the preparation is favored by its transport behavior, including dependence of water transport from isotonic Ringer fluid on the presence of glucose in the medium, water absorption against an osmotic activity difference, uphill glucose movement, and chloride impoverishment.

The purposes of the present paper are to describe in greater detail the relationship between fluid movement and pressure, and to discuss the possible relevance of the in vitro results to the role that increased subepithelial tissue pressure may play in vivo in conditions characterized by high rates of secretion.
same pressure difference between mucosal and serosal pressure was established by elevating the pressure on one fluid or diminishing that on the other below atmospheric. The membranes were not supported. “Mucosal pressure” will denote the excess pressure on the mucosal fluid and vice versa for “serosal pressure.”

No distinction will be made between fluid and water transport ($J_F$), the value for which, in any case, was determined by changes in weight of the fluid in the membrane (inner) chamber. The values will be expressed as milliliters per hour per membrane of 6.4 cm² area. Absorption will be taken as positive in sign and secretion, negative. These terms are used to indicate direction only, without regard to mechanism.

Chemical Methods

Sodium was determined by flame photometry, and glucose by the alkaline ferricyanide method, both by standard Technicon AutoAnalyzer procedures. Chloride was estimated by the method of Cotlove et al. (3).

RESULTS

Relationship Between Fluid Transport and Pressure

The relationship found between fluid transport ($J_F$) and pressure is shown in Fig. 1.

In the absence of a pressure difference $J_F$ was absorptive at a rate of about 1 ml/hr. This rate is similar to those reported for other intestinal preparations in vivo and in vitro (11). When mucosal pressure was increased to 22 cm H₂O, there was no marked effect on $J_F$ for either jejunal or ileal membranes. By contrast, when serosal pressure was raised to only some 2–6 cm H₂O, $J_F$ was reduced to zero, and further elevations in the serosal pressure produced secretion of fluid (negative $J_F$). The secretion could reach values of over 10 ml/hr at serosal pressures of about 20 cm H₂O. The secretory values for the jejunal membranes tend to be higher than for those from the ileum. The mean value for the slope of a straight line fitted to the secretory points is about 0.4 ml/hr per cm H₂O serosal pressure. In the region between ΔP = 0 and ΔP = −4 cm H₂O ($J_F = 0$), $L_p$ is about 0.25 ml/hr per cm H₂O.

Relationship Between Water and Solute Transport

a) Sodium and chloride. Figure 2 shows the relationship between $J_F$ and the rate of Na movement ($J_{Na}$) for both jejunal and ileal membranes. Figure 3 is the corresponding plot for chloride movement ($J_{Cl}$).

During fluid absorption, the slopes of lines fitted to the points are close to the concentrations in the bathing fluids. The intercepts of the lines (fitted by least squares) relating $J_{Cl}$ to $J_F$ are somewhat different for the two regions of the intestine. For the jejunum at zero fluid movement there was some net entry of chloride into the mucosal fluid, whereas for the ileum $J_{Cl}$ was zero. In the case of Na, the intercept is close to zero for both regions.

The relationships plotted apparently did not change with reduction in $J_F$ or reversal of its direction by serosal pressure. In both directions the points fall on the same straight lines.

b) Other solutes. As previously reported (12), the kinetics of urea transport by this preparation could be reasonably well predicted by a combination of diffusion and convection. The model formulated on this basis applied equally well to either net fluid absorption or secretion produced by serosal pressure (several of the experiments included in the present paper are the same as those in the previous one). In particular, the relationship between urea movement and fluid secretion was quite...
consistent with the occurrence of solvent drag through channels considerably wider than the urea molecule.

The relationship between glucose transport and fluid secretion is also of this type, as illustrated by the results for experiments in which the mucosal fluid was in the inner compartment and contained glucose at a concentration of 1,000 mg/100 ml, whereas the large volume serosal fluid contained 500 mg/100 ml. Any net transport of glucose into the mucosal fluid would thus be uphill. Figure 4 shows that the normally occurring mucosal disappearance of glucose (positive $J_G$) was reversed to net mucosal appearance (negative $J_G$) by pressure-induced fluid secretion. Moreover, the form of the relationship is consistent with filtration of fluid carrying glucose with it convectively without much sieving. For the purposes of comparison a line has been drawn for the results which would have been obtained had the serosal fluid been delivered to mucosal fluid by a pipette (simple bulk flow). Discrepancies between observation and the line for this limiting case could be due to a number of factors including diffusion, utilization, active absorption, and a sieving coefficient less than 1.0 when the rate of fluid secretion is small.

Inulin was similarly transported uphill under these conditions whereas normally the preparation shows very low permeability to this molecule even in the absorptive direction. Evans blue, ferritin, and finally erythrocytes could be seen entering the mucosal fluid when pressures about 10 cm H$_2$O were maintained. Somewhat higher pressures could produce obvious leaks promptly.

**DISCUSSION**

It is apparent that in the dog mucosal preparation the effects of pressure on fluid transport are greatly different depending on whether the excess pressure is applied to the mucosal or serosal side of the membrane.

**Excess Mucosal Pressure**

The simplest interpretation of the fact that mucosal pressures up to 22 cm H$_2$O did not demonstrably affect $J_v$ is that the mucosa has a low hydraulic permeability ($L_p$). Values for $L_p$ have been estimated from osmotic data for this and other preparations, including the human small intestine in vivo (9), on the assumption that effective osmotic pressure per se does not affect
hydraulic permeability. These values are such that no detectable effect would have been expected from the hydrostatic pressures employed here.

Data on the effects of mucosal distending pressure on fluid movement have been reported for the Fisher and Parsons rat intestinal preparation (6) and for the Smyth and Taylor modification of this preparation (16, 23). In the distension pressure range of 0-20 cm H₂O only the values of Lee are at hand, but for higher pressures values from all three series of observations are available and are in good agreement, as shown in Fig. 5. At any given pressure Jᵥ has been normalized by expressing it as a percentage of the value at 20 cm H₂O pressure; the great majority of points in the figure represent average values and not single experiments. According to Fig. 5, Jᵥ rises some fourfold as the distension pressure increases from slightly greater than zero to about 10 cm water. With further augmentation of the pressure, Jᵥ increases less rapidly, about 40% between 20 and 70 cm H₂O.

The corresponding observations on the dog mucosa are for 0-22 cm H₂O and differ from the above in that they do not suggest a definite pressure dependence. The apparent difference may be related to the circumstance that the dog preparation consisted of a mucosal membrane stretched across a glass tube whereas the rat preparations were suspended segments of whole intestinal wall.

In the rodent preparations, Jᵥ is absent or much reduced when glucose or O₂ is omitted from the bathing fluids, or when phlorizin or commonly used metabolic inhibitors are added. Under these conditions mucosal pressure does not produce or enhance Jᵥ. The same is true of the canine mucosal membrane (11). Since in these circumstances Jᵥ would presumably be passive, such observations are consistent with low values for hydraulic permeability (Lp). Unless the Lp increases when the preparation is in the more favorable bathing fluids, then the rise in Jᵥ with distension in the rat preparations must be attributed to factors associated with active water movement, either driving forces (e.g., metabolism, coupling) or resistances (structure of the membrane, broadly considered).

**Excess Serosal Pressure**

There is little question but that the secretory fluid transfer produced by serosal pressure either widens

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**FIG. 4.** Relationship between the rate of glucose and fluid movement as measured by changes in the mucosal bathing fluid of membranes exposed to excess serosal pressure. Appearance (apparent secretion) is negative in sign.

**FIG. 5.** Composite results for the relationship between Jᵥ and pressure for rat in vitro preparations. Observations of the various series have been normalized by setting Jᵥ at P = 20 cm H₂O at 100. The dashed line represents probable extrapolation according to results of Wilson (29) for the hamster.
existing channels or opens new ones, or both, to increase \( L_p \). In the case of everted segments of hamster small intestine studied in vitro, Wilson (31) found that small excess hydrostatic pressures on the serosal fluid had relatively large effects on water transport. A pressure of only 4 cm \( H_2O \) sufficed to prevent absorption and higher pressures caused net secretion. Thus, the dashed line in Fig. 5 is the presumed extension into the serosal pressure region of the relationship between \( J_V \) and pressure for the rodent preparation. Serosal pressures greater than 10 cm \( H_2O \) sometimes produced small perforations which permitted the passage of Evans blue dye. The dog mucosal membrane showed apparently identical behavior. However, even in the absence of such obvious leaks through which Evans blue, ferritin, and even red blood cells can pass, the kinetics of urea and glucose transport are reasonably predictable from diffusion and simple bulk flow with little or no sieving.

The question arises as to why a change in the structure of the membrane leading to perforation can occur so much more readily from excess serosal pressure than from mucosal pressure. We think the answer is most probably related to the asymmetry of the morphology of the lateral contact between the epithelial cells, as discussed by Parsons (19). It is also possible that defects in the epithelium left by the continuously shedding cells are kept open and widened when tissue pressure is high.

The factors involved in the decrease of \( J_V \) to zero as the serosal pressure is increased from 0 to about 4 cm \( H_2O \) should be the same as those producing secretory flow at higher pressures except that the direction of net fluid flow is not reversed, and obviously detectable perforations do not occur. Presumably, active fluid absorption and secretory filtration are proceeding in parallel and are equal to one another when \( J_V = 0 \). But aside from the possibility that active absorption or secretion may be affected by serosal pressure, the question arises as to the extent to which the two streams are independent. In one limiting case the two would be completely independent, in which case a fluid circuit would be present; in the other, the two streams would cancel each other as in a common channel, in which case when \( J_V = 0 \), there would be no convective movement.

Two implications of a fluid circuit are that a) with the same solute concentration on the two sides of the membrane, net solute transport would occur when \( J_V = 0 \) if the streams have different sieving coefficients; and b) that with different solute concentrations on the two sides and equal sieving coefficients in the two streams there should be an elevation of the apparent contribution of diffusion to net solute transport. The latter implication follows from the fact that the absorptive and secretory flows would carry different solute concentrations; hence, when \( J_V = 0 \) both diffusion and the fluid circuit would contribute to net solute transport proportional to the concentration difference and be formally taken as diffusive. Actually it was found that both the apparent diffusive permeability and sieving coefficient of urea were similar for active fluid absorption and pressure induced secretory flow (12). Therefore, this symmetry in kinetics argues against independence of the two types of fluid transfer. Consistent with this inference are the data for Na and Cl transport shown in Figs. 2 and 3. In particular, when fluid absorption is reduced toward zero by small serosal pressures, the relationships between solute and fluid movements are those one would expect in the absence of the pressure (2, 4, 32). The difference between the intercepts for the jejunum and ileum for chloride are no doubt due to the greater tendency for luminal bicarbonate to exchange for chloride in the jejunum. The evidence against independent flows from the electrolyte data is not as strong as from the observations on urea because the Na and Cl concentrations of the two bathing fluids were the same and the sieving coefficients may well be the same (about 1.0) in both kinds of flow. Interaction between the two flows to produce, as found, solute kinetics like that through a single channel does not require the routes for active absorption and secretory filtration to be identical, but only that some portion be shared in common.

An interesting implication of the urea results is that the effects of serosal pressure are general. For if the contrary were true, i.e., were serosal pressure producing secretory filtration in relatively few sites, active absorption should have been taking place in the rest of the membrane to establish a fluid circuit. Among the possibilities responsible for such a generalized effect are a) that active fluid transport is inhibited by small serosal pressures with larger pressures producing an increase in \( L_p \); b) that serosal pressure breaks intercellular seals rather uniformly so that fluid delivered to the intercellular space by active absorption would be forced back into the lumen; c) that a small number of localized breaks in the epithelium occur in most villi (shedded cells?) and the hydrodynamic resistances in vascular and interstitial channels of the stroma are such that actively absorbed fluid is driven back into the lumen by serosal pressure. An electron microscopic attempt to locate the postulated channels, thus far unsuccessful, is being continued.

**Width of Intercellular Channels to Account for Rates of Secretory Filtration**

It is of interest to estimate the half-width of the intercellular channels required for the observed rates of the presumed secretory filtration, roughly 0.4 ml/hr per cm water for 6.4 cm² membrane \((1.8 \times 10^{-8} \text{ in cgs units})\). The equation for the half-width for filtration at rate \( J_V \) through slits of width \( w \), total length \( L \), and thickness \( \Delta x \), across which there is a pressure difference \( \Delta P \), is (18):

\[
\frac{w}{2} = \frac{1}{2} \left( \frac{12 \eta \Delta x J_V}{L \Delta P} \right)^{1/2}
\]

\( \eta \) is the viscosity of the filtrate, assumed to be water \((7 \times 10^{-5} \text{ dynes-sec/cm}^2)\). From measurements of the
surface geometry of mounted membranes, the following mean values were obtained for the jejunum: number of villi, 870/cm²; area per villus, 1.76 \times 10^{12} \text{cm}^2; nonvillus area, 0.56 \text{cm}^2. The total mucosal area per 6.4 \text{cm}^2 of mounted membrane thus becomes about 100 \text{cm}^2. If the mucosal surfaces of the epithelial cells are assumed to be squares of sides 10 \mu m, L of the intercellular spaces is about 2 \times 10^{-6} \text{cm}; the value is less than 20% higher if the cells are hexagons of side 10 \mu m. If \Delta x is assigned a value of 30 \mu m (about the length of the cell), equation 1 then gives a half-width of 65 \AA and the area occupied by the slits (wL) is about 0.25% of the total mucosal area. If \Delta x = 0.3 \mu m (about the length of the tight junctions), the corresponding values are 14 \AA and 0.06%. These values for half-width are similar to those estimated for capillaries (18). If the total serosal area of the small intestine is 2,000 \text{cm}^2 (26), the rate of 0.4 ml/hr per cm H_2O for the membrane would become 125 ml/hr for the whole intestine, or over 1 liter/hr for a \Delta P of only 10 cm H_2O.

Although the assumption of uniform spaces between cells is highly questionable, these calculations do not seem to us to reduce the plausibility of the intercellular route for the secretory fluid and solute movement.

Possible Relevance of the Observations to In Vivo Intestinal Function

It has repeatedly been suggested or assumed that secretory filtration occurs in the intestine in vivo, particularly in various diarrheal states. It is the theory of Wells that filtration is the process occurring in intestinal secretion (27-30); more recently Fordtran has discussed the matter with reference to the pathogenesis of cholera (7). Excess serosal pressure in in vitro preparations would be analogous to excess mucosal interstitial pressure in vivo; and the question arises as to the possible relevance to the in vivo situation of the increased hydraulic permeability produced by small elevations of serosal pressure.

There are observations in the literature which appear to support the applicability of this aspect of the in vitro results to the in vivo situation. Wells (30) reported that the proportionality constant between rate of fluid secretion by a canine intestinal loop and intraluminal pressure was increased when the pressure in the veins draining the loop was raised by partially compressing them. This result is very suggestive that the elevation of the apparent \( L_p \) was contingent on increased tissue pressure. Secretion produced by distension pressures of the order of the diastolic arterial blood pressure (70-110 cm H_2O) (5, 13) is also interpretable in this way.

Wells (30) and Shields and Code (22) found in dogs that when the portal vein pressures were increased to more than 40 cm H_2O, blood entered the intestine. Undoubtedly, the tissue pressure was also elevated and the mechanism of the passage of the blood cells across the epithelium may well have been similar to that in vitro. It further would appear probable that the decrease in net fluid absorption proceeding to the net secretion associated with raised portal vein pressures (see below) also corresponds to the in vitro results with excess serosal pressure. It is, moreover, interesting that Shields and Code abandoned the procedure of intermittent raising and lowering the portal vein pressure because, after increasing the portal pressure for only 15 min, transport of water did not return to control values for a matter of hours. This kind of result is highly suggestive of slowly repairable morphological damage such as the disturbance of an intercellular epithelial seal.

It is relevant that secretory filtration appears to occur in the stomach. From a study of the composition of the alkaline fluid secreted by canine gastric mucosa in response to acetylcholine and the luminal pressure required to prevent this secretion, Altamirano has concluded that the secretion is essentially an ultrafiltrate of plasma plus additions from the mucous cells (1). The most direct measurements of the pertinent pressure relationships in the villi are those of Königes and Ottó (15), made in the cat. Since the pressure in the central lacteal averaged 24.5 mm Hg (32 cm H_2O), the tissue pressure should have been at least this high; the colloid osmotic pressure of thoracic duct lymph averaged 10.4 mm Hg. Therefore, as Wells pointed out (30), a driving force for secretory filtration presumably existed. However, these observations also provide a possible objection to the in vivo applicability of the in vitro results. Even with a luminal pressure as high as 20 cm H_2O, the excess pressure at the transepithelial serosal pole would be 12 cm H_2O, sufficiently large to cause perforation of the hausture or dog epithelium in vitro, if serosal pressure and tissue pressure are not very different. Possibly the intestine in vivo may have a significantly higher resistance to perforation, or the values of Königes and Ottó may not represent a sustained normal situation.

(It appears from the results of Fig. 1 that any degree of serosal pressure reduces the rate of absorption (Jv). If this reduction is due to secretory filtration, one must conclude that even small serosal pressures (P_s) not only increase \( L_p \) but are accompanied by a net passive driving force across the epithelium in the secretory direction. Presumably this secretory driving force is (P_T - P_M - \sigma P_F) so that when serosal pressure is increased by perhaps only 1 cm H_2O, (P_T - P_M) > \sigma P_F. Here P_T and \pi_T are the subepithelial tissue fluid hydrostatic and colloid osmotic pressures, P_M is the mucosal fluid hydrostatic pressure, and \sigma is the reflection coefficient of the tissue fluid colloids. P_T would be greater than P_s as long as \( J_v \) is absorptive. Perhaps when \( P_s - P_M = 1 \text{ cm H}_2\text{O} \), P_T - P_M may be several cm H_2O. We do not know \pi_T for these membranes, but the same factors which increased \( L_p \) would tend to decrease \sigma. In any event, it appears likely that the product, \sigma P_F, should be less than P_T - P_M with slightly elevated serosal pressures in order to reduce the net fluid absorption.)

By the use of D_2O, measurements have been made of the unidirectional fluxes of water in three conditions in which the normally occurring isotonic small intestinal fluid absorption changes to net secretion: elevated portal
ven pressure (22), simple intestinal obstruction (21), and experimental cholera (24). If filtration into the lumen were in fact occurring in these abnormal states, one might predict increased values for the unidirectional flux rate from blood to lumen. Actually, however, contrary to this prediction, it was found in all these studies that net absorption could change to net secretion without an increase in the unidirectional secretory flux. However, this discrepancy between prediction and observation is not necessarily incompatible with the idea that filtration was the mechanism responsible for the net secretion. The basis of this statement is given in the following section: it is essentially that total unidirectional flux measurements of water do not distinguish between diffusive and convective contributions to the total flux, so that a decrease in diffusive flow could mask an increase in convective secretory flux.

Interpretation of Net and Unidirectional Fluxes of Water During Intestinal Absorption or Secretion

We believe there is sufficiently common misunderstanding of this subject to justify the following discussion at this point.

Each unidirectional flux rate for water, as traced by D$_2$O, may be considered as consisting of three components: passive diffusion, passive convection, and active transport. The distinction between passive and active processes here is with respect to whether the driving force is external (as by osmotic or hydrostatic pressure differences) or metabolically created within the membrane (as by active sodium transport). Convection may be variously defined as "macroscopically observable" or "bulk" flow which can produce solvent drag. The active component will be assumed to be convective, as seems likely. It will be further assumed that the three components are independent of one another and in the normal reference state with isotonic isosmotic luminal fluids, there are present only bidirectional passive diffusion and active (convective) absorption (Fig. 6A). The kinetics of D$_2$O absorption by the dog intestine are reasonably consistent with these assumptions (17). In Fig. 6A, arbitrary units are assigned to the diffusive and convective fluxes the relative values of which are in accord with observation. The active absorptive flow is assigned a value of 10 arbitrary units; the two unidirectional diffusive rates are assigned equal values (40 units) because the water activity is the same in the luminal fluid and plasma. Given this equality, the only way in which net secretion can arise is by the onset of convective secretory flow, either active or passive (as by filtration due to raised tissue pressure). Net secretion or absorption cannot be due to diffusion. The total absorptive flux is thus 50 units; total secretory flux, 40 units; and net absorption, of course, 10 units.

Figure 6B illustrates that net secretion could be produced without increased total passive secretory flux due to filtration if it is accompanied by a suitable decrease in the two necessarily equal diffusive fluxes. As compared with the normal situation (Fig. 6A), the new situation pictured would have a reduced total unidirectional flux from lumen to blood of 30 (20 diffusion + 10 convection) and an unchanged total flux from blood to lumen of 40 (20 diffusion + 20 convection). Consequently one would be arithmetically justified in saying that the change from net absorption to net secretion was due entirely to a reduction in absorption from 40 to 30 with secretion remaining constant at 40. But in terms of mechanism, the reversal of direction of net transport was due solely to the onset of convective secretion. The actual mechanism has simply been masked by the reduction in the rate of diffusion.

Another possibility is that the condition causing secretory filtration and the reduction of passive diffusion also inhibited active absorption. One might then observe the results shown in Fig. 6C. In this case the interpretation on the basis of the total unidirectional rates would be again that since total secretion was still 40, the mechanism of net secretion was a reduction in absorption only, whereas in fact it was a combination of a reduction in...
convective absorption from 10 to 0 and the added secretory convection of 10.

It also is pertinent to point out that the actual calculations of unidirectional flux rates with D₂O in the lumen assume, among other things, that the transepithelial D₂O concentration is zero. Even in normal dogs this assumption is invalid to some extent (10); and the invalidity should be greater in abnormal circumstances characterized by reduced transcapillary exchange, such as decreased capillary blood flow or increased tissue fluid volume. The effect of invalidity is to reduce the calculated quantity of D₂O leaving the lumen by diffusion. This would lead to an underestimate of the unidirectional rate in both directions, and to this extent mask any increase in total flux in the secretory direction due to filtration.

In summary, the available unidirectional flux data cannot be used with any assurance as highly accurate measures of total unidirectional fluxes of water; and, even if accurate, they may be misleading as a test of whether increased filtration into the lumen occurred.

These comments bear on interpretations of unidirectional flux rates in relation to fluid circuit mechanisms for intestinal absorption. The proposal of Ingraham, Peters, and Visscher (14) postulates simultaneous convective streams in the two directions. It is evident, however, that the reality and quantitative importance of such circuits cannot be inferred from two-way movement of isotopic water, no matter how large, because the movement opposite to the direction of the net transport could be entirely diffusive. It is also incorrect, as has recently been done (8), to imply that unidirectional rate and diffusional rate are synonymous.

From the values of total unidirectional fluxes occurring in a segment of intestine, it can be estimated that in normal animals a volume of water equal to the plasma volume enters and leaves the intestines several times a day. From this it is sometimes argued that with such a large quantity of water being secreted, relatively small fractional reductions in the rate of its absorption would lead to copious diarrhea, even without an increase in the rate of secretion. The arithmetic is correct but again is misleading mechanistically. Only the convective contribution to the total unidirectional secretory flux, not its total value, is subject to fecal loss. In going from the normal to the diarrheal state, the component of fecal loss due solely to failure to absorb secreted intestinal fluid would at most be the normal rate of convective secretion, not the total secretory flux.

For purposes of illustration, let us assume again that the normal state in the small intestine is represented by Fig. 6A with the arbitrary units now denoting liters per day. We will further assume that 11 liters enter the intestines through the pylorus, bile duct, and pancreatic duct, of which 10 liters (50 minus 40 liters) would thus be absorbed and 1 liter delivered to the large intestine as potential fecal loss. If a 10% decrease in this lumen to blood flux (from 50 to 45 liters) should occur, it is argued that one then would find that net absorption would decline to 5 liters; and 6 liters instead of 1 liter would enter the large intestine as potential fecal loss. However, if the diminished absorptive flux were due to a reduction in the diffusive component, the decrease could not occur without a simultaneous and equal decrease in the blood-to-lumen flux. The net absorption would remain at 10 liters. On the other hand, if the decrease were in the convective component of the absorptive flux, a 50% reduction would be necessary for a 5-liter decrease (not a 10% one). The maximum addition to fecal loss by complete inhibition of absorption would be 10, not 50 liters. Similarly, there could be no increase in potential fecal loss by changes of any magnitude in the diffusive secretory flux.

On this basis, it would follow that the changes from net absorption to net secretion, whenever found in closed intestinal loops (as in the case of raised portal vein pressures, intestinal obstruction, and cholera), would in themselves be very suggestive evidence for increased secretory convective flow, possibly filtration.

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