Drug Transport V: Mechanism of Potassium-Ion Inhibition of Passive Transfer of Solutes across Everted Rat Intestine

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Abstract □ In previous reports, it was shown that the inclusion of certain cations and sugars in the drug–buffer solution, or other alteration in the basic buffer solution, that results in net tissue fluid uptake also produces a marked reduction of passive solute transfer across the everted rat intestine. Since the extent of inhibition of intestinal transfer under any given condition varied considerably from one drug to another, it was decided to examine the effect of potassium ion (K^+), a particularly pronounced inhibitor, on the intestinal transfer of a series of solute molecules of widely different permeability characteristics. The quantitative replacement of Na^+ by K^+ in a Krebs bicarbonate buffer resulted in some degree of transfer inhibition of every compound studied. An inverse relationship was found between the degree of transfer inhibition and permeability. The intestinal transfer of poorly permeable compounds such as bromthymol blue or eosine blue was virtually abolished in the presence of K^+. A high degree of correlation was noted between percent inhibition and molecular weight of solute molecules, suggesting that the most important parameter influencing the extent of inhibition of transfer is the molecular size of the compound. A mechanism is proposed whereby polar compounds are assumed to traverse the isolated, everted rat intestine *via* intercellular channels existing between adjacent mucosal epithelium cells. When the intestine is exposed to buffer solutions causing tissue fluid uptake, a portion of the fluid penetrates the mucosal cell, causing swelling of the cell and producing a narrowing of the apical portion of the intercellular channel.

Keyphrases □ Drug transport, everted rat intestine—potassium-ion inhibition □ Potassium ion—mechanism of passive-transport inhibition □ Tissue fluid uptake relationship—drug transport

Previous reports in this series showed that various cations present in a drug–buffer solution can have a marked influence on passive solute transfer across the everted rat intestine. In the presence of potassium (1, 2), there is a significant reduction in the mucosal-to-serosal transfer of riboflavin, salicylate, and sulfanilamide. Other cations, including NH_4^+, guanidine$^+$, and Li^+, produce varying degrees of transfer inhibition of these molecules. Several recent literature reports supported these observatons on the influence of K^+ on passive solute transfer. Caldwell *et al.* (3, 4) showed that the passive transfer of digoxin and ouabain across the everted rat intestine is significantly reduced in the presence of a K^+ buffer. In addition, there was direct verification of the authors' observations on the influence of K^+ on salicylate transfer by Benet *et al.* (5).

Inclusion of certain other components in the drug–buffer solution or alteration in the basic buffer solution produces the following effects on transfer: (*a*) glucose and xylose reduce solute transfer, whereas the inclusion of mannitol has no effect (6); and (*b*) a hypotonic buffer solution significantly decreases solute transfer, while a hypertonic buffer exerts no influence on transfer (7).

A common characteristic of all buffer components causing an inhibition of solute transfer is their influence on tissue fluid uptake. There is a direct, quantitative relationship between the percent inhibition of transfer and the extent of tissue fluid uptake under these varying conditions (6). However, the extent of inhibition of transfer produced by any given buffer component varies from one drug to another. Thus, consistently, the transfer of riboflavin is inhibited to a significantly greater extent than is the transfer of salicylate or sulfanilamide. Since these differences cannot be attributed to the buffer component, they must be related to the permeability characteristics of the molecule.

Accordingly, it was decided to examine the effect of K^+ on the intestinal transfer of a series of solute molecules of widely different permeability characteristics and to attempt to correlate the biologic parameters with appropriate properties of the molecules. It was anticipated that this approach would prove useful in understanding the mechanism(s) of passive drug transfer across the isolated intestine.

EXPERIMENTAL

Intestinal Transfer Studies—Intestinal transfer rates were determined using a modification of the method of Crane and Wilson (8). Preparation of the everted rat intestine and determination of transfer rate were discussed in a previous report (2). The following briefly summarizes the procedure and notes any departures from the usual methods.

Male, Sprague-Dawley rats, weighing about 250 g., were fasted 20–24 hr. prior to the experiment. Water was allowed *ad libitum*. After severing the intestine at the pyloric junction, the first 15 cm. of proximal intestine was discarded and the following 20 cm. was divided into two 10-cm. segments after eversion. The proximal 10-cm. segment was designated Segment 1. The normal buffer solution (Na^+ control) was a modified Krebs bicarbonate buffer[1], pH 7.4. The K^+ buffer was prepared by quantitatively replacing the sodium salts of the control buffer with the corresponding potassium salts. In each experiment, 2 ml. of buffer was placed inside the sac (serosal solution); the entire preparation was placed into approximately 100 ml. of buffer solution containing the drug (mucosal solution), which was continually gassed with O_2–CO_2 (95:5 v/v). The drugs studied are listed in Table I.

In any given experiment, the mucosal and serosal solutions were identical in composition except for the presence of the drug in the mucosal solution. Usually, the serosal compartment was sampled every 10 min. during the entire 2-hr. experiment. The entire serosal volume was removed at the sampling time. Two milliliters of buffer solution was then introduced into the serosal compartment as a rinse, immediately removed, and added to the previous sample. Finally, another 2-ml. portion of buffer was placed into the serosal compartment and withdrawn at the next sampling interval. In

[1] KCl, 5 m*M*; KH_2PO_4, 1 m*M*; $NaHCO_3$, 26 m*M*; and NaCl, 122 m*M*.

Table I—Compounds Selected for Intestinal Transfer Studies

Compound	Molecular Weight	pKa	Mucosal Concentration, mg./ml.	Assay Procedure
Aniline (A)	93.1	4.7	0.1	*Reference 9* (see text)
Antipyrine (AP)	188.2	1.4	0.5	*Reference 10* (see text)
Benzocaine (*p*-ethylaminobenzoate) (B)	165.2	2.8	0.1	*Reference 9*
Bromthymol blue (BTB)	624	—	1.0	In 0.1 *N* NaOH at 620 nm.
Eosine blue (EB)	624	—	0.5	In 0.1 *N* NaOH at 520 nm.
Methyl orange (MO)	327	—	0.25	In 0.1 *N* HCl at 510 nm.
2-Pyridine aldoxime methiodide (PAM)	264	—	0.4	In 0.1 *N* NaOH at 335 nm.
Riboflavin (R)	376	10.2	0.02	*Reference 11*
Salicylamide (SAM)	137	8.3	1.0	*Reference 12* as modified in *Reference 13*
Salicylate (SA)	137	3.0	2.0	*Reference 12* as modified in *Reference 14*
Sulfanilamide (S)	172	10.4	0.1	*Reference 9*

two cases, eosine blue and bromthymol blue, the compounds traversed the intestinal preparation so slowly that the serosal solution was sampled only at 60 and 120 min. Each serosal sample was assayed for drug content using the methods noted in Table I.

Aniline concentration was determined using a previously described method (9) with the following modification. It was found that the rate of coupling to produce a stable color was much too slow using the concentration of reagent originally suggested. Consequently, the concentration of Marshall's reagent [*N*-(1-naphthyl)ethylenediamine·2HCl] was increased to 500 mg./ml. This modification produced a stable color, which was read spectrophotometrically after a 2-hr. waiting period. The increased concentration of reagent had no effect on the maximal absorbance reading or on the time for stable color development for a compound (sulfanilamide) which is readily assayed using the usual reagent concentration. Antipyrine was also determined according to a previous report (10), but absorbance readings were made exactly 20 min. after the addition of $NaNO_2$. The initial concentration of the respective compounds in the mucosal solution, as noted in Table I, remained essentially constant throughout the experiment due to the large volume of the mucosal solution. Moreover, due to frequent sampling of the serosal compartment, in all but two cases the serosal drug concentration never exceeded 13% of the mucosal concentration. The maximum serosal concentrations for aniline and salicylamide were 20 and 30%, respectively, of the mucosal drug concentration.

Comparison of the permeability characteristics of the various solutes was based on the mucosal-to-serosal clearance of each compound over the 1st hr. of study. Clearance (milliliters per hour) was calculated by dividing the cumulative amount of solute transferred in 1 hr. in the Na^+ control buffer by the mucosal solute concentration. The effect of K^+ on intestinal transfer was quantified in terms of the percent inhibition of transfer over a 1-hr. period:

$$\% \text{ inhibition} = 100(1 - \frac{\text{1 hr. cumulative transfer } K^+}{\text{1 hr. cumulative transfer in } Na^+}) \quad \text{(Eq. 1)}$$

Determination of Extracellular Tissue Volume—The extracellular volume of intestinal tissue was determined in various buffer solutions. The marker used was ^{14}C-carboxylinulin[2] (specific activity 2.0 mc./g., 97% pure). The inulin was used as obtained from the supplier. Levi (15) reported that inulin space measurements using inulin from this manufacturer provides consistently good, reproducible estimates of extracellular volume. The inulin was dissolved in distilled water, and dilutions made from this stock were taken to prepare various tagged buffer solutions. Snyder's scintillation fluid (16) was used (20 ml./scintillation vial) for all samples. The composition of this fluid is:

0.3000 g.	1,4-bis[2-(5-phenyloxazolyl)]benzene[3]
7.0000 g.	2,5-diphenyloxazole[3]
100.0 g.	naphthalene[3]
	dioxane to make 1 l.

All materials other than dioxane were of scintillation grade.

The rat intestine was everted as described previously, and the first 15 cm. was discarded. Five or six 5-cm. segments were prepared from each intestine and made into sacs (17). A ligature was tightly tied around one end of the segment, and a long blunt needle attached to a 1-ml. syringe was introduced into the open end of the segment. Another ligature was loosely tied around the needle, and 1 ml. of the appropriate buffer soluton (*i.e.*, the same solution as the mucosal buffer), previously warmed at 37°, was placed into the segment. The ligature about the needle was then tightened and the needle was withdrawn. All sacs were then placed into a beaker containing the tagged inulin buffer solution at 37°, which was gassed with an O_2–CO_2 mixture (95:5 v/v).

Extracellular space measurements were made under two experimental conditions. The tagged inulin was present initially only in the mucosal solution, or it was present in the mucosal solution as well as in the 1 ml. of serosal solution in the sac. The sacs were removed after 60 min. incubation in the mucosal solution and gently, but thoroughly, rinsed with normal saline. The sac was cut open at one end and the serosal fluid was permitted to run out. Three snips of tissue (about 100 mg. each) from each segment were then placed into individual, previously tared scintillation vials and the vials were immediately capped. Several samples (0.1 ml.) of the mucosal inulin buffer solution were also taken and placed into vials containing scintillation fluid. Each tared vial was then weighed to determine the final wet weight of tissue. One milliliter of solubilizer[4] was added to each vial, and the vials were placed into a water bath maintained at 45–50° to hasten the digestion process. After the tissue was in solution (generally after 3–4 hr.), the vials were cooled to room temperature and scintillation fluid was added. All samples were counted for 10 min. at 10,000 c.p.m. in a liquid scintillation spectrometer[5].

Quenching of radiation due to the presence of tissue was corrected for by use of an internal standard (^{14}C-toluene). Generally, there was a 12% loss of counting efficiency due to tissue quenching. If samples were read within a day of preparation, unusually high counting rates were obtained, apparently due to an interaction between the solubilizer[4] and the scintillation fluid. After preparing blank vials (*i.e.*, tissue + solubilizer + scintillation fluid

[2] Lot. No. 511-021, New England Nuclear Corp., Boston, Mass.

[3] Amersham-Searle Co., Arlington Heights, Ill.

[4] NCS solubilizer, Amersham-Searle Co., Lots F-7230 and F-7290.

[5] Packard Tri-Carb, model 2002, Packard Instrument Co., Downers Grove, Ill.

Table II—Clearance and K^+ Inhibition of Transfer

Compound	Clearance, ml./hr.[a]	Percent Inhibition of Transfer in K^+[b]
Bromthymol blue	0.016[c] (46)	86[c]
Digoxin	0.100[d]	67[d]
Eosine blue	0.126 (43)	91
Methyl orange	0.380 (12)	75
Riboflavin	0.540 (12)	56
Sulfanilamide	0.570 (14)	34
Pyridine aldoxime methiodide	0.720 (11)	52
Salicylate	0.800 (18)	27
Antipyrine	1.720 (4)	24
Benzocaine	3.240 (6)	13
Salicylamide	3.420 (4)	16
Aniline	4.600 (4)	6

[a] Based upon cumulative amount transferred across Segment 1 after 1 hr. in the Na^+ control buffer. Represents the average of five experiments (four for sulfanilamide). Parenthetic values denote the coefficient of variation expressed as a percentage. [b] Based upon cumulative amount transferred across Segment 1 after 1 hr. in the K^+ buffer. Represents the average of five experiments (four for sulfanilamide). [c] Based upon cumulative amount transferred after 2 hr. [d] Calculated from *Reference 4.*

with no added radioactivity), it was found that if samples were read 3 days after preparation, this background counting rate was reduced to a sufficiently low level to be subtracted from all samples. Since this background rate represents at most 1% of the activity present in the sample, there is little error introduced after correcting for this level of activity. As standard procedure, therefore, all samples were counted 3 days after preparation.

Extracellular tissue volume was calculated from the following relationship:

$$\text{extracellular space (ml./g.)} = \frac{\text{c.p.m./g. tissue}}{\text{c.p.m./ml. mucosal inulin solution}} \quad \text{(Eq. 2)}$$

RESULTS

Solute Properties and K^+ Inhibition of Transfer—Table II lists clearance values for each compound based upon the 1-hr. cumulative transfer in the Na^+ control buffer, and the percent inhibition of transfer in the K^+ buffer based on the 1-hr. transfer data. There appears to be a distinct relationship between the clearance value and the extent of inhibition of transfer in the presence of the K^+ buffer; as the clearance value increases (*i.e.*, as permeability increases), the percent inhibition of transfer decreases. Thus, bromthymol blue and eosine blue, which have the smallest clearance values, are inhibited to the greatest extent. The most permeable compounds, benzocaine, aniline, and salicylamide, are inhibited the least. Since these three compounds exhibit the greatest permeability, it is possible that the rather small, constant level of inhibition of transfer produced by K^+ is not a true measure of the extent of inhibition. That is, since these compounds readily traverse the membrane, the rate-limiting step in the overall transfer process may be the rate of movement of the molecule through the mucosal solution to the mucosal surface (*i.e.*, bulk diffusion rate-limited transfer). If this were true, then the rate of appearance of the drug in the serosal solution would be a measure of diffusion through the mucosal solution and not transfer across the membrane. Such a situation could conceivably account for the low level of effect of K^+ on the transfer of these permeable compounds. If, in fact, the overall transfer process was bulk diffusion rate limited, then the transfer rate should be sensitive to stirring effects in the mucosal solution. This possibility was examined by providing rapid stirring in the mucosal solution by means of a small magnetic stirrer. The transfer rates of both aniline and salicylamide were independent of stirring rate; as a result, it may be concluded that transfer across the membrane is not rate limited by bulk diffusion.

Figure 1 illustrates the inverse relationship between the extent of inhibition of transfer and clearance. The clearance of digoxin

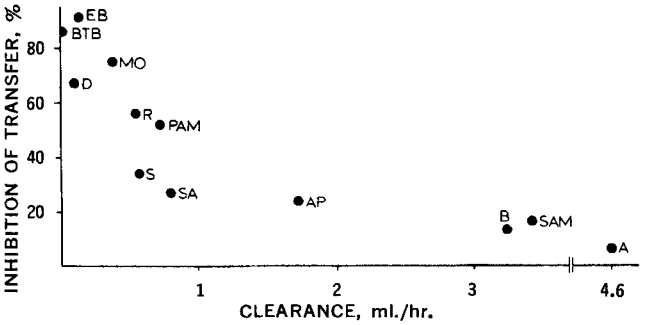

Figure 1—*Relationship between percent inhibition of transfer after 1 hr. in the K^+ buffer and the clearance after 1 hr. in the Na^+ control buffer. Each point represents the average of five experiments (four for sulfanilamide) using Segment 1. Key: see Table I.*

and the percent inhibition of transfer of digoxin in the presence of K^+ were calculated from data in a recent literature report (4). While the degree of inhibition produced by K^+ is clearly related to the permeability of the compound, it was desirable to develop this relationship further.

Figures 2 and 3 are log–log plots of percent inhibition of transfer and of clearance in the Na^+ buffer, respectively, as a function of molecular weight. A least-squares line was fitted to all points in each graph. In both cases, a highly significant relationship is observed. The plot of percent inhibition *versus* molecular weight yields a correlation coefficient of 0.884 and indicates that the larger the molecule the greater the extent of inhibition of transfer. The plot of clearance in the Na^+ buffer *versus* molecular weight also yields a high correlation coefficient (0.880) and suggests that, at least for the compounds studied, clearance decreases with increasing molecular weight.

Extracellular Space of Intestinal Tissue in the Presence of Various Buffer Solutions—The relationship between tissue fluid uptake and the inhibition of transfer by various buffer components was developed previously (2, 6). This relationship suggests that the fluid uptake produced by a given inhibitor may be responsible for the alteration of the transfer process. Since fluid uptake by the intestinal tissue may localize either within cells or in extracellular space, an attempt was made to determine where this fluid localizes in the tissue by measuring extracellular space.

Initially, attempts were made to determine extracellular space by placing closed everted intestinal sacs into a tagged inulin solution (inulin present only in the mucosal solution). Although a value of approximately 22% was obtained for this space in the Na^+ control

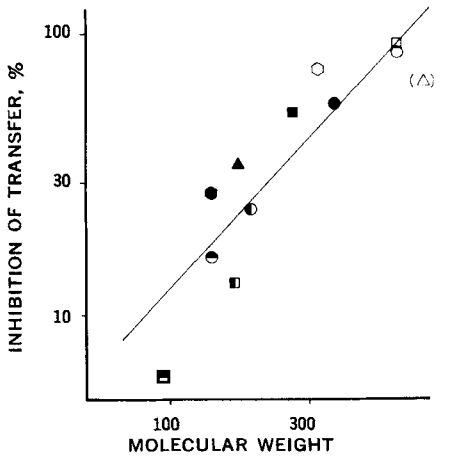

Figure 2—*Relationship between percent inhibition of transfer after 1 hr. in the K^+ buffer and the molecular weight. Values for percent inhibition are the average of five experiments (four for sulfanilamide) using Segment 1. A least-squares regression line was drawn through all points. Key: ●, salicylate; ■, 2-pyridine aldoxime methiodide; □, eosine blue; ○, methyl orange; ●, riboflavin; ▲, sulfanilamide; ◐, antipyrine; ○, bromthymol blue; ▬, aniline; ◓, salicylamide; ◧, benzocaine; and △, digoxin.*

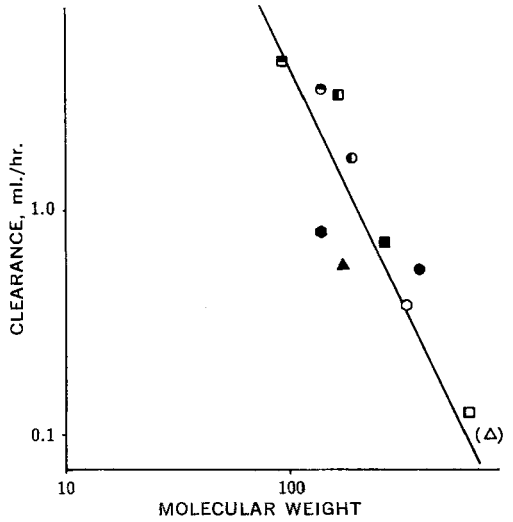

Figure 3—*Relationship between the 1-hr. clearance in the Na^+ control buffer and the molecular weight. Clearance values represent the average of five experiments (four for sulfanilamide) using Segment 1. A least-squares regression line was drawn through all points. Key: same as Fig. 2.*

buffer, which is consistent with certain literature reports (18, 19), the great variability precluded a meaningful comparison of extracellular space values under different conditions. As a result, inulin was placed into both mucosal and serosal solutions. This improved the reproducibility of the results, as shown in Table III. Although the value of 49% obtained in the Na^+ control buffer seems unusually large, a recent literature report (19) suggested that this value is reasonable.

Preliminary examination of the extracellular space data based on final tissue weight suggests a clear relationship between extracellular space and inhibition. The last four buffers listed in Table III (250 m*M* glucose, hypotonic, K^+, and 25 m*M* glucose) markedly inhibit the transfer of various polar compounds and significantly decrease extracellular space. On the other hand, tromethamine, mannitol, and hypertonic buffers have little effect on transfer and are either without effect on or actually increase extracellular space. However, there is some controversy as to whether extracellular space values should be calculated on the basis of final tissue weights (19). Considering the equation used to calculate extracellular space, it is clear that any factor which tends to increase or decrease the weight of the tissue will give an apparent change in extracellular space regardless of where the fluid is localized. Since it has been found that all buffer solutions which inhibit solute transfer also produce an increase in tissue weight, the value for tissue weight will be larger than the control. This results in an apparent decrease in extracellular space.

Table III—Total Extracellular Space of Rat Intestinal Tissue in the Presence of Various Buffer Solutions

Buffer Solution[a]	Percent Extracellular Space Based upon Final Tissue Weight[b]	Percent Extracellular Space Based upon Initial Tissue Weight[c]
Na^+ control	49 ± 6 (14)	52
Hypertonic	65 ± 4 (15)	61
Tromethamine	54 ± 6 (15)	56
Mannitol	44 ± 3 (15)	45
250 m*M* glucose	41 ± 4 (15)	47
Hypotonic	40 ± 5 (15)	44
K^+	34 ± 4 (15)	40
25 m*M* glucose	33 ± 3 (15)	40

[a] As reported in *References 2, 6,* and *7.* [b] Represents the average ± standard deviation of the mean. The number of tissue pieces used to determine extracellular space are given in parentheses. [c] Represents the experimentally measured extracellular space of the control corrected for tissue changes due to fluid uptake in the various buffer solutions.

Furthermore, the decrease in space will be related to the extent of fluid uptake. When one corrects the extracellular space values shown in Table III for water uptake, *i.e.*, when one calculates extracellular space based on initial rather than final tissue weights, it can be seen that there is little change in extracellular space with different experimental conditions. A recent report by Jackson and Cassidy (20) also demonstrated no relationship between tissue fluid uptake by the rat intestine and extracellular space and is consistent with the present findings.

DISCUSSION

From the previous reports in this series (2, 6, 7), it seems clear that some parameter related to tissue fluid uptake is responsible for the observed decrease in solute transfer in the presence of various buffer solution components. A plausible mechanism that would rationalize previous experimental observations as well as the apparent dependency of the magnitude of transfer inhibition on the size of the solute must take into account the route of movement of the compound across the membrane. With respect to solute movement across multicellular membranes, one may consider at least three possible routes: (*a*) movement *via* a lipid route (*i.e.*, through the cells *via* the lipid membranes), (*b*) transport through aqueous-filled channels existing between adjacent cells (*i.e.*, intercellular spaces), and (*c*) transport *via* pores existing within the cell membrane in contact with the mucosal bathing solution. The least probable route would be the latter. It is doubtful that there would be discontinuities in the cell membrane of sufficient size to accommodate compounds of a molecular weight in excess of 100 (21).

With respect to solute translocation *via* a lipid route, one would expect that this pathway would be limited solely to compounds having sufficient nonpolar characteristics. In the present study, it was found that those compounds which could be considered lipid soluble are also the most permeable molecules. It would seem most plausible that these compounds traverse the intestine essentially by penetration across the cell membrane, and it is the transfer of these compounds which is least inhibited by K^+. This observation, as well as several other experimental findings, suggests that K^+ and the other inhibitors are preferentially affecting another route of solute movement across the intestine which is distinct from the lipid pathway.

One possibility, therefore, to explain the results of the inhibition of solute transfer is to assume that polar compounds penetrate the isolated, everted rat intestine *via* an intercellular route. The mechanistic explanation for inhibition of transfer would be based upon the following reasoning. Under normal conditions (*i.e.*, in the presence of the Na^+ control buffer), polar molecules penetrate the isolated intestine by movement along intercellular channels existing between adjacent mucosal cells, whereas nonpolar molecules would primarily use a lipid route. When the intestine is placed into a buffer solution causing tissue fluid uptake, a portion of the fluid penetrates the mucosal cell, causing swelling of the cell. This increase in cell volume produces an expansion of adjacent cells, resulting in a narrowing of the intercellular channels and decreasing the effective diameter of these channels.

Apparently inconsistent with this possibility is the observation that there is no substantial decrease in the extracellular space in the presence of those materials causing a reduction in solute transfer. This finding may not be inconsistent, however, based upon the morphology of the intestinal epithelium. The intercellular channels have an appearance very much like a funnel. At the luminal surface of the intestine, the intercellular spaces are rather long and narrow and gradually funnel-out approximately two-thirds the way down the epithelial cell. Beyond that point, these channels are very large and this region probably represents virtually all of the mucosal extracellular volume. Several studies with rat intestine illustrated that the basal portion of the intercellular channels can be widely distended. Tomasini and Dobbins (22) observed distention of these channels using the electron microscope, and Dobbins (23) showed these spaces to be approximately 200 Å wide. Palay and Karlin (24) reported average distances of 95–120 Å, with some distances as great as 50–200 nm. separating adjacent mucosal cells. Thus, these channels beyond their narrow regions should be able to accommodate relatively large molecules. As a result, any narrowing of the intercellular channels at their closest points would have little

effect on the total extracellular space measured experimentally, although it may have a profound effect on the relative width of these narrow channels. Support for such a mechanism includes the following experimental observations:

1. Those materials whose transfer is not dependent upon use of intercellular channels should be affected little, if at all, by buffer components producing a decrease in transfer of polar solutes. In fact, the observation has been made that those compounds which are lipid soluble and readily permeable and whose transfer most likely involves movement *via* a lipid route are affected the least by K^+ (6–16% inhibition).

2. All buffer components that result in an inhibition of transfer increase tissue fluid uptake (2, 6, 7). A portion of this fluid uptake produces cellular swelling in the rat intestine (20, 25, 26). Other investigators showed that fluid uptake in a variety of different tissues produces cellular swelling at the expense of extracellular spaces (27–30), although this has not been shown to be the case for intestinal tissue both in the present and in a previous literature report (20). In addition, those materials that have little effect on transfer do not increase tissue fluid uptake.

3. It would be expected that if the effective diameter of narrow intercellular spaces was decreased due to cellular expansion, the larger the molecule the greater would be the inhibition of transfer. Such a relationship exists between molecular weight and inhibition. In addition, under control conditions there is an inverse relationship between molecular weight and clearance, indicating a size dependence for transfer consistent with restricted movement through rather narrow channels.

There are several conflicting reports in the literature with respect to the accessibility of intercellular channels at the luminal membrane surface. Several investigators (23, 24, 31, 32) feel that the tight junction at the luminal border between adjacent epithelial cells presents a real barrier to solute accessibility to the intercellular channels. This conclusion is primarily based upon electron microscopic examination of intestinal tissue. Clarkson (33) developed equations to analyze salt and water transport across the isolated rat ileum, and he concluded that there are at least two distinct pathways for the movement of these materials: one having a large diameter (compared to the hydrated ionic radii of Na^+, K^+, and Cl^-) located extracellularly and permitting passive transport of salt and water, and the other having a small diameter, located intracellularly and associated with active transport processes. The author also suggested that epithelial cells extruded from the luminal surface leave "gaps" which partially determine the passive permeability of the intestinal tissue, although these "gaps" may be transient and occupy a small fraction of the total mucosal surface area.

In vivo measurements of the rat ileum (34) based upon mannitol diffusion indicate the presence of aqueous channels having radii in the order of 30–40 Å, whereas the equivalent mean pore radius of the epithelial cell membrane is only 4 Å (35). More recently, Loehry *et al.* (36) studied the permeability of the rabbit small intestine *in situ* by measuring the plasma-to-lumen and lumen-to-plasma clearances of various water-soluble molecules over the 60–33,000 molecular weight range and of ^{125}I-labeled polyvinylpyrrolidone molecules over the 8000–80,000 molecular weight range. The authors reported that all substances studied pass through the small intestinal wall and that an inverse relationship exists between permeability and molecular weight identical to that found in the present study. Moreover, the permeability pattern is virtually identical whether diffusion occurs from plasma to lumen or from lumen to plasma. These findings require one to postulate the existence of aqueous channels with much greater diameters than those proposed for the epithelial cell membranes. It would appear, based on the present finding as well as on several literature reports, that an aqueous intercellular route across the mucosal epithelium plays a very significant role in the intestinal transport of polar molecules both in isolated systems and *in vivo*.

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