Differential Permeability of the Proximal and Distal Rabbit Small Bowel

ALLAN ROSS, ALLEN W. RUBIN, and JULIUS J. DEREN

From the Department of Medicine, Gastrointestinal Section of the University of Pennsylvania, School of Medicine, Philadelphia, Pennsylvania 19104

ABSTRACT The permeability of the proximal and distal rabbit intestine for two to six carbon polyhydric alcohols was compared. Intestinal segments were mounted in chambers that permitted the measurement of the unidirectional flux across the brush border membrane. For both proximal and distal intestine, the permeability for a series of polyhydric alcohols decreased with increasing size. The proximal intestine was more permeable for four, five, and six carbon polyhydric alcohols than distal intestine. This regional permeability difference can be attributed to variations in the permeability characteristics of the brush border specifically. The uptake of alcohols was nonsaturable and was not inhibited by phlorizine or *n*-ethylmaleimide. The results are compatible with the concept that the brush border membrane has properties similar to artificial porous membranes and that the equivalent radius of the pores of the proximal intestine exceeds that of the distal gut.

INTRODUCTION

The proximal and distal small bowel differ in morphology (1), enzyme activity (2), and transport properties (3-5). In addition, evidence has been presented for a difference in the pore size of the proximal and distal small bowel in the human (6), although no difference has been observed in the dog (7).

The studies described herein (*a*) demonstrate that rabbit proximal small bowel is more permeable to four, five, and six carbon polyhydric alcohols than the distal intestine, and (*b*) indicate that this regional permeability difference arises from variation in the permeability characteristics of the brush border membrane. These data are consistent with the concept that the equivalent radius of the pores of the proximal gut exceeds that of the distal intestine.

Received for publication 13 March 1972 and in revised form 24 April 1972.

METHODS

Measurement of uptake by rabbit intestine. The uptake of polyhydric alcohols was measured by the technique described by Schultz, Curran, Chez, and Fuisz (8). Rabbit bowel was opened along the mesenteric border and impaled onto the lower section of a plexiglass chamber above a piece of filter paper moistened in Krebs-Ringer bicarbonate buffer (9). The serosal surface faced downward onto the filter paper and the mucosal surface was exposed to a compartment to which 1 or 2 ml of Krebs-Ringer bicarbonate buffer solution was added. The entire chamber was placed in a constant temperature apparatus at 37°C and attached to a gas source which allowed for continuous stirring of the mucosal solution by humidified 5% CO_2–95% O_2. After a 30 min equilibration period the buffer was removed and 1 ml of test solution containing ^{14}C-labeled polyhydric alcohol and tritium-labeled methoxy inulin was added. At the end of 30 sec the test solution was rapidly removed and the tissue was washed with ice-cold isotonic mannitol. The exposed 1.13 cm^2 of tissue was excised with a steel punch, washed briefly again in ice-cold mannitol, and extracted overnight in 0.1 normal nitric acid. Portions of the incubation solution and the tissue were counted in a liquid scintillation counter at settings appropriate for simultaneous counting of ^{14}C and tritium. The tritiated inulin corrected for adherent mucosal fluid not removed by the washing procedure. Results were expressed as permeability coefficients which have the dimensions of centimeters per second. Preliminary experiments demonstrated that the rate of uptake to be linear for 60 sec and thus the entry of ^{14}C label represents the unidirectional flux of the polyhydric alcohol across the brush border unaffected by intracellular metabolism or back diffusion (8).

Measurement of mucosal surface area relative to serosal surface area. The method employed was similar to that described by Fisher and Parsons (10). Segments of distal and proximal small bowel were stretched slightly and impaled onto a piece of wood and placed in Bouin's fixative for 24 hr. After routine dehydration, clearing and paraffin embedding, sections were cut along the longitudinal axis of the small bowel. The sections were stained with hematoxylin and eosin and projected with a microprojector onto white paper, and tracings of the mucosal and serosal were surfaces obtained. The length of each surface was measured with a map measurer and results were expressed at the ratio of mucosal to serosal surface.

Materials. Erythritol-^{14}C (U), galactitol-1-^{14}C, D-man-

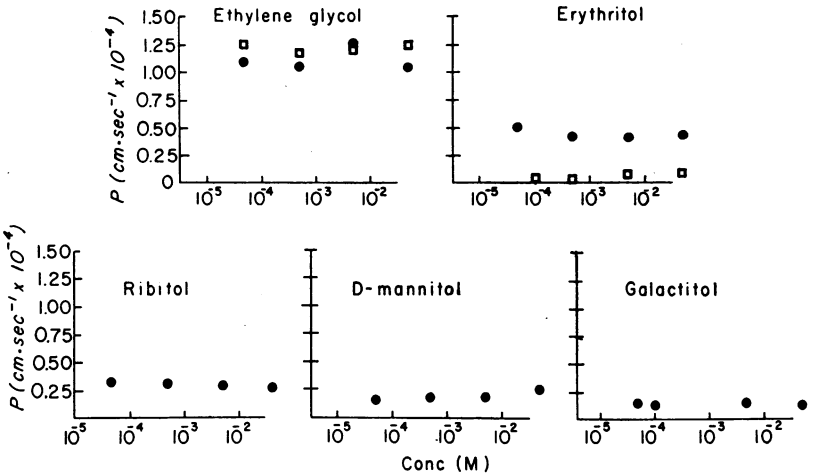

FIGURE 1 Permeability coefficients (P) of a series of polyhydric alcohols across the brush border of proximal (○) and distal (□) rabbit small intestine at various concentrations. The permeability for each polyhydric alcohol over the indicated range was obtained on a single segment of intestine. The number of segments studies was six for ethylene glycol, six for erythritol, three for ribitol, six for D-mannitol, and three for galactitol.

nose-^{14}C (U), D-xylose-^{14}C (U), and D-mannitol-1-^{14}C were purchased from Amersham/Searle, Arlington Heights, Ill., and ethylene glycol-1,2-^{14}C, inulin-methoxy-^{3}H and ribitol-1-^{14}C from New England Nuclear Corp., Boston, Mass. Erythritol, galactitol, D-mannose, D-mannitol, and ribitol were purchased from Pfahnstiehl Lab. Inc., Waukeegan, Ill., and D-xylose from Fisher Scientific Co., Fairlawn, N. J.

RESULTS

Movement of ethylene glycol, erythritol, ribitol, mannitol, and galactitol across the brush border of rabbit intestine. As shown in Fig. 1, the permeability coefficients obtained for each polyhydric alcohol was constant over a concentration range of 5×10^{-5} to 5×10^{-2} M. The permeability coefficient obtained with the two carbon polyhydric alcohol, ethylene glycol, was similar for both upper and lower intestine. The permeability of the intestine for the four carbon polyhydric alcohol, erythritol, was less than that for ethylene glycol. In addition, the upper intestine displayed a significantly greater permeability for erythritol than the lower gut. The entry of the five (ribitol) and six (mannitol and galactitol) carbon polyhydric alcohols into distal intestine was minimal, ranging from zero permeability (distribution of ^{14}C label between medium and tissue not different than the distribution of methoxy-inulin) to a permeability coefficient of less than 0.05 cm·sec$^{-1} \times 10^{-4}$. In contrast, significant entry of the five and six carbon polyhydric alcohols was consistently observed with proximal intestine with permeability coefficients of 0.30 for ribitol, 0.21 for D-mannitol, and 0.14 for galactitol. These results are summarized in Fig. 2 in which the permeability coefficients observed for each polyhydric alcohol for both proximal and distal intestine are plotted against the molecular radius of the test compound.

The effect of phlorizin and n-ethylmaleimide (NEM) on the uptake of polyhydric alcohol by proximal rabbit intestine. Table I shows that neither phlorizin at a concentration of 5×10^{-4} M nor n-ethylmaleimide at a concentration of 10^{-3} M significantly inhibited the uptake or ribitol or D-mannitol.

Comparison of the permeability of proximal intestine

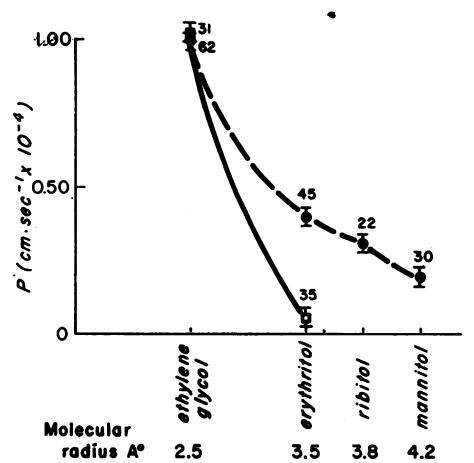

FIGURE 2 Permeability coefficients (P) of polyhydric alcohols across the proximal (○) and distal (□) rabbit small intestine. Since the permeability coefficients remained constant over the concentration range studied, all values obtained over the course of these studies were averaged. The values given represent the mean values of all studies performed ±SEM.

TABLE I

*Effect of Phlorizin and n-ethylmaleimide (NEM) on Mannitol and Ribitol Permeability**

Test compound	Inhibitor	No.‡	Permeability coefficient: Alone	Permeability coefficient: With inhibitor	P
			$cm \cdot sec^{-1} \times 10^{-4}$		
Ribitol	Phlorizin	7	0.58±0.04§	0.56±0.03	> 0.05
Mannitol	Phlorizin	2	0.11	0.11	
Ribitol	NEM	6	0.37±0.06	0.39±0.05	> 0.05
Mannitol	NEM	6	0.30±0.04	0.26±0.03	> 0.05

* The concentration of phlorizin was 5×10^{-4} M, NEM 10^{-2} M, and ribitol and mannitol 10^{-4} M.
‡ Number of studies.
§ Values given are the mean values ±SEM.

for five and six carbon polyhydric alcohols and five and six carbon aldoses. As shown in Table II both ribitol and D-mannitol entered the proximal rabbit intestine at a more rapid rate than D-xylose and D-mannose. Furthermore, a considerable portion of the xylose entry (50%) was inhibited by phlorizin (data not shown).

TABLE II

Comparison of the Uptake of Five and Six Carbon Polyhydric Alcohols to Five and Six Carbon Ring Compounds by Proximal Rabbit Intestine

Test substance*	No.‡	Permeability coefficients	P
		$cm \cdot sec^{-1} \times 10^{-4}$	
Ribitol	5	0.56±0.03§	< 0.05
D-xylose		0.20±0.04	
D-Mannitol	5	0.28±0.09	< 0.05
D-Mannose		0.05±0.10	

* All compounds were present at 1×10^{-4} M concentration.
‡ Number of paired studies.
§ The values given are mean values ±SEM.

The ratio of mucosal to serosal surface area of proximal and distal rabbit intestine. The mucosal to serosal surface area ratio as estimated from segments examined under light microscopy are shown in Table III. There was 25% more mucosal surface area per unit serosal surface area for proximal as compared to distal intestine.

TABLE III

The Ratio of Mucosal to Serosal Surface Area of Proximal and Distal Rabbit Intestine

Intestinal segment	No.	Mucosal to serosal surface area	P
Proximal	8	8.8±0.5*	< 0.01
Distal	8	6.7±0.9	

* Values given are mean values ±SEM.

DISCUSSION

Studies in the intact human have shown that the filtration coefficient for water is greater for the proximal gut than the distal intestine (6). In addition, small water soluble compounds exert less than their theoretical osmotic pressure when placed in the proximal gut but their Staverman reflection coefficients approach one in the distal intestine (6). Based on an analogy to artificial porous membranes these data were interpreted to indicate that the radius of the membrane pores was larger in the proximal gut than in the distal intestine. These interpretations are quite reasonable but would appear to have several major limitations. Firstly, as pointed out by Fordtran, Rector, Ewton, Soter, and Kinney (6), when studying the intact human a number of permeability barriers are imposed between lumen and blood and thus one could not ascribe differences in pore size specifically to anyone of these barriers. Secondly, the movement of fluid from lumen to blood across the gut may traverse different pathways or may display different characteristics than fluid movement in the opposite direction. In fact, striking asymmetry of bulk flow of water across the vitro frog intestine has been recently demonstrated (11). Since Fordtran et al. (6) measured bulk fluid movement by placing hypertonic solutions in the gut lumen their data may reflect regional differences in bulk movement of water from blood to lumen rather than differences in the bulk movement of water from lumen to blood. Furthermore, both the hydraulic conductivity and the permeability to small water soluble molecules may be altered in the presence of hypertonic solutions (12). Thirdly, the possibility that movement of the test molecules across the gut was a result of a carrier-mediated transfer mechanism was not vigorously evaluated. The presence of a carrier-mediated movement for urea and erythritol in the proximal gut and its absence from the distal gut would account for the failure of these two compounds to exert their theoretical osmotic pressure in the proximal intestine. Carrier-mediated movement of erythritol and urea have been demonstrated in red blood cells (13, 14). The studies described herein clearly demonstrate that the proximal rabbit intestine displays greater permeability for four, five, and six carbon polyhydric alcohols than the distal intestine and that this regional permeability difference arises from variation in the permeability characteristics of the brush border membrane specifically. These results are similar to those reported in the intact human in whom the diffusion rates for urea, xylose, and

erythritol were lower in the distal than in the proximal human small bowel (15).

In the in vitro preparation used in this study a segment of intestine 1.13 cm^2 is exposed to the transported compound. The precise absorbing surface area is not shown since the surface area of the mucosal border is increased to a variable degree by macroscopic (villi) and microscopic (microvilli) projections. A difference in the absorbing surface area of the proximal and distal gut exposed to the incubating solution is, however, unlikely to explain the differences in permeability. Firstly, the ratio of mucosal to serosal surface area in proximal and distal intestine, measured on hematoxylin- and eosin-stained light microscopic sections differed by only 25%. Secondly, ethylene glycol, a smaller water-soluble compound whose entry kinetics were compatible with simple passive diffusion, entered both the proximal and distal gut at a similar rate. Assuming entry through aqueous channels ("pores") these data would indicate that the total effective pore area available is the same in proximal and distal gut (16). Thirdly, the V_{max} and K_m for the transfer mechanism of glucose, a process clearly localized to the brush border, was similar in both areas of the rabbit small intestine (unpublished observation).

In order to determine whether the decrease in permeability with increasing size was related to the slower diffusion of the larger compound in the unstirred layer surrounding the microvilli, the results with proximal gut were plotted as shown in Fig. 3. If diffusion through the unstirred layer were responsible for the decrease in permeability, the products of the permeability coefficient and the square root of the molecular radii would have been constant. As shown, this product decreased with increasing molecular size so that factors other than diffusion through the unstirred layer was responsible for the decreased permeability with increasing molecular size.

Although the differences in permeability of the proximal and distal intestine do not appear to arise from variations in absorbing surface area, the results do not permit an unequivocal choice as to whether this difference results from variation in the pore radius or as a result in the presence of a special membrane transfer mechanism in the proximal gut. The absence of saturation kinetics is compatible with diffusion through pores although a carrier-mediated process with low affinity may similarly fail to show saturation kinetics. A search for competitive inhibitors may differentiate between these two modes of transfer. For that reason, the effect of phlorizin, a potent inhibitor of the prominent glucose transfer mechanism (17), and NEM, which inhibits a number of carrier-mediated processes in the gut (18) were evaluated for their effect upon ribitol and mannitol transfer. As shown in Table I, no significant inhibition

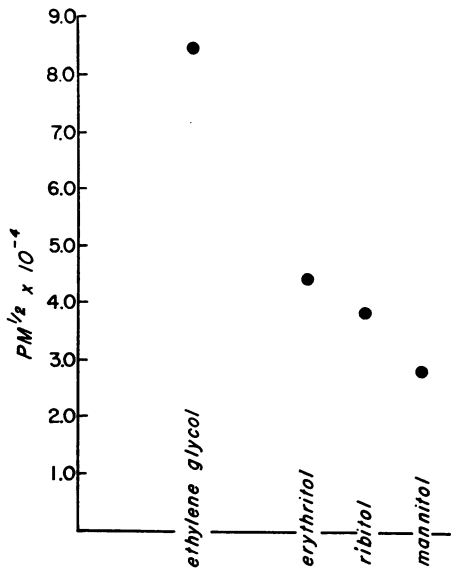

FIGURE 3 Variation of $PM^{\frac{1}{2}}$ in proximal intestine with molecular size. P represents the permeability coefficient ($cm \cdot sec^{-1} \times 10^{-4}$), and $M^{\frac{1}{2}}$ the square root of the molecular weight.

of ribitol and mannitol was observed in the presence of NEM or phlorizin. This observation, of course, does not exclude carrier-mediated transfer since the carriers may not have significant affinity for the arbitrarily chosen inhibitors. Another approach that was employed to differentiate between these two mechanisms was to compare the movement of five and six carbon polyhydric alcohols to other water-soluble compounds of similar molecular dimensions. For this purpose ribitol transfer was compared to xylose and mannitol movement to mannose. Viscometric data indicate that five and six carbon polyhydric alcohols have molecular dimensions similar to their five and six carbon aldoses (19). The five and six carbon polyhydric alcohols entered proximal intestine at a more rapid rate than xylose and mannose. In fact xylose entry was significantly inhibited by phlorizin and presumably a portion of this entry occurred via the glucose transfer mechanism rather than through membrance pores. This observation of greater permeability of ribitol and mannitol as compared to xylose and mannose suggests that molecular size is not the sole determinant of movement. It is not clear, however, whether these data indicate that the polyhydric alcohols do indeed react with a membrane carrier to facilitate their movement or whether differences in the structure of xylose and mannose apart from size alone restrict their movement through the pores.

The failure to observe saturation kinetics or competitive inhibition with either phlorizin of NEM favors the passive movement of these compounds across the brush border but does not unequivocally exclude a carrier-

mediated movement. It is suggested that the polyhydric alcohols penetrate through aqueous channels which are thought to be interspersed with the lipid matrix, a concept first proposed by Collander and Barlund (20) and Höber and Höber (21) and recently by Solomon (22). The data would indicate that the equivalent radius of the pores of the proximal intestine is greater than those of the distal intestine and therefore permit the entry, although at a restricted rate, of five and six carbon polyhydric alcohols. The shape of the curve relating permeability coefficients to molecular radius conforms to the shape of the curve observed when studying the movement of molecules of graded size across artificial porous membranes (16) and thus further suggests that we are observing this mode of transfer. Furthermore, if a carrier-mediated mechanism were present it would be unlikely for interaction with the carrier to be determined solely on the basis of the size of the interacting solute. An alternate explanation for these observations is derived from an analysis of membrane permeability by Davson and Danielli (23) and recently expanded upon by Stein (24). According to this concept, fixed water-filled channels are not present in the membrane. Water-soluble compounds move across cell membranes by entrance into and diffusion across the lipid component of the membrane proper. The transported molecule must thus first detach itself from the surrounding water molecules in the external aqueous environment, diffuse through the lipoidal component of the membrane by simple diffusion, and finally the molecule must acquire sufficient energy to detach from the lipid layer and enter the aqueous phase in the cell interior. According to this concept the decreasing permeability with the increasing size of the polyhydric alcohols would not be related to size but rather to the increasing number of hydroxyl groups which can form hydrogen bonds with water and thus would require greater activation energy to leave the aqueous phase before entering the lipid component. In this concept of membrane permeability the partition coefficient between the oil (membrane) and water phase is the important determinant of membrane permeability. In order to explain the difference in permeability for polyhydric alcohols of the upper and lower gut one would have to postulate a difference in the composition of the brush border membrane from these two areas. The brush border of the upper intestine would have to have a composition which would lead to a partition coefficient which would favor the penetration of the water soluble polyhydric alcohols. Interestingly, regional variations in the composition of purified brush borders have recently been observed (25).

ACKNOWLEDGMENTS

During the tenure of this work, Mr. Ross (first year medical student, University of Pennsylvania School of Medicine) was supported as a Summer Research Fellow by a General Research Grant No. 5145-10, subcontract No. 3, from the U. S. Public Health Service to the University of Pennsylvania School of Medicine. This work was supported by National Institute of Research Grant AM-13089 and Training Grant AM-5462.

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