PARACELLULAR CHANNEL CHARACTERIZED BY NON-ELECTROLYTE PERMEATION THROUGH
THE COLONIC MEMBRANE OF THE RAT

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The mucosa-to-serosa permeability of non-electrolytes through the stripped colonic rat mucosa was examined in a Ussing-type chamber. The permeation clearances for inulin (12-15 Å radius) to erythritol (3.2 Å radius) increased linearly with the increase in their free diffusion coefficients. On the other hand, the clearances of glycerol, thio-urea and urea of less than 3 Å radius increased in excess of what would be expected considering the above linearity. This suggests that there are large pores that do not restrict diffusive flow and small pores of 3 Å radius or greater that restrict diffusion in the paracellular channel. The trans-cellular permeation also occurred in a tracer efflux experiment with urea preloaded in colonic stripped mucosa. It was not ruled out that the higher permeability of the non-electrolytes less than 3 Å in radius was due to a transcellular route in parallel with the paracellular one.

KEYWORDS - paracellular route; pore radius; transcellular route; rat colon; diffusion coefficient; non-electrolyte

INTRODUCTION

There are two permeation routes, transcellular and paracellular, in the intestinal membrane.^{1,2)} We have reported an increase in the paracellular permeation resulting from an increase in the pore size effected by absorption promoters as a mechanism for poorly absorbed drugs in rat colon.³⁾ A control value of approximately 8 Å radius was obtained by equivalent pore theory⁴⁾ and the promoters increased it to 13-15 Å. The

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equivalent pore theory was derived under the assumption that all the pores are uniform and circular. The equivalent pore radius thus may give us information on the average physical property of a complexed biological membrane.⁴⁾ On the other hand, two pore sizes, 60 and 7 Å, were reported in the ileal rat membrane based on the relation between volume flow generated by neutral probe molecules and their molecular radii.⁵⁾ So also for the colonic membrane, the pore distribution with various sizes may be expected.

In this study, the colonic permeability to water-soluble non-electrolytes with different molecular radii, which are expected to permeate predominantly through the paracellular route, were examined to obtain the substantial pore radii and compare them with the equivalent pore radius described above.

MATERIALS AND METHODS

Materials - ^{14}C -Urea, ^{14}C -thiourea, ^{14}C -glycerol, ^{14}C -mannitol, ^3H -polyethylene glycol 900 (PEG 900), ^3H -inulin were purchased from New England Nuclear (Boston, Mass). ^{14}C -Erythritol was obtained from Amersham (Bucks, England). All other reagents were of analytical grade or better.

Membrane Permeation Experiment - Wistar male rats (250 ± 20 g) were used in preparing colonic sheets. The mucosa stripped of underlying muscle was mounted as a flat sheet in the Ussing chambers (0.75 cm^2 exposed surface area).^{6,7)} On both the mucosal and serosal sides, 11 ml of Ringer solution ($1.2 \text{ mM NaH}_2\text{PO}_4$, 125 mM NaCl , 5 mM KCl , 1.4 mM CaCl_2 , 10 mM NaHCO_3 and 2 g/l D-glucose , 280 mOsm/kg) was added, and to the mucosal solution $1\text{--}3 \text{ }\mu\text{Ci}$ of ^{14}C - or ^3H -labeled non-electrolytes were added. Following a 10-min time lag, the amounts of permeation from the mucosal to serosal sides were obtained for 60 min at 37°C under constant bubbling with $95\% \text{ O}_2 - 5\% \text{ CO}_2$. The apparent membrane permeation clearance ($P_{\text{m,app}}$) ($\mu\text{l/min/cm}^2$) was obtained as (permeation rate)/(mucosal concentration kept constant during the experiment). The $P_{\text{m,app}}$ values were corrected for the unstirred water layer to obtain real membrane permeation clearance (P_{m}) as shown previously.³⁾

Efflux Experiment - The isolated rat colon segment (about 150 mg), stripped of underlying muscle and rinsed with Ringer solution, was incu-

bated for 2 h in 2 ml Ringer solution containing 1 μCi ^{14}C -urea at 37°C under constant bubbling with 95% O_2 - 5% CO_2 . Following the loading period and a preliminary 10-min rinse in 20 ml of label-free Ringer solution, the tissue segment was taken through a series of 10-min rinses in 1.5 ml label-free Ringer solution. Every 10 min, 1-ml samples were taken from each of the rinsing solutions and efflux amounts (efflux rates, dpm/min) were determined. Finally the tissue segment was swabbed with damp filter paper and the wet weight determined within 0.1-mg accuracy.

Intracellular Volume - Following the permeation experiment with inulin, the colonic mucosa (0.75 cm^2) was weighed as described in the efflux experiment and then was incubated for 2 h in 0.5 ml label-free Ringer solution to extract inulin.⁶⁾ The extracellular volume was obtained as (extracted inulin amount)/(mucosal inulin concentration kept constant during the experiment). The intracellular volume (V) ($\mu\text{l}/\text{cm}^2$) was obtained from the difference between the tissue segment weight (volume) and the extracellular volume.

Assay - Experimental samples, 1 ml serosal solutions for the membrane permeation or 1 ml rinsed solutions for the efflux, were mixed with 10 ml scintillator cocktail (Scintisol EX-H, Dojin Lab., Kumamoto, Japan) and ^3H and ^{14}C activities were counted by liquid scintillation counter (Aloka 903, Tokyo, Japan). The decrease in the counting efficiency resulting from quenching was automatically corrected by the external standard source.

Free Diffusion Coefficients (D) and Molecular Radii (α) of Non-electrolytes - The D and α values of all non-electrolytes are listed together with their molecular weights in Table I.

RESULTS AND DISCUSSION

The relationship between P_m and D is shown in Fig. 1. For inulin, PEG 900, mannitol and erythritol with diffusion coefficients of less than $8.6 \times 10^{-6}\text{ cm}^2/\text{s}$, i.e., above about 3 Å radii (Table I), the relationship was linear and the ordinate intercept was not significantly different from zero ($p > 0.05$). Glycerol, thiourea and urea had P_m values higher than the expected linear values. The permeation of nonelectrolyte showed that the linearity may occur via an unrestricted diffusion in the paracellular route. The higher permeability for glycerol, thiourea and urea may

TABLE I. Molecular Weights (MW), Free Diffusion Coefficients (D) and Molecular Radii (α) of Non-electrolytes Used in Membrane Permeation Experiment

	MW ^{a)}	D(x10 ⁻⁶ cm ² /s) ^{b)}	α (Å) ^{b)}
Urea	62	12.4	2.0 ^{c)}
Thiourea	78	10.9	2.6
Glycerol	94	9.9	2.9
Erythritol	124	8.6	3.2
Mannitol	184	7.1	3.9
PEG 900	900	3.2	— ^{d)}
Inulin	5000	1.3	12-15 ^{d)}

a) The molecular weights were those of the radiolabelled molecules; those of PEG 900 and inulin were approximate values.

b) From reference 8. c) From reference 9. d) From reference 10.

be due either to an additional paracellular route or to the transcellular route. The additional paracellular route is regarded as the restricted one which is less available for the larger molecules of more than 3 Å radius. Water net flux was not detected in this permeation experiment through the small membrane area (0.75 cm²), so the solvent drag effect was neglected. The exact pore radius cannot be determined by the above results only, but the value responsible for the restricted permeability must be close to 3 Å to allow glycerol, thiourea and urea to permeate this route. Thus, it was smaller than the 7 Å reported for the smaller pore in the ileal membrane⁵⁾ and our equivalent pore radius (8 Å) in the colonic membrane.³⁾ According to the pore theory, however, the restricted diffusion within cylindrical pores requires a greater radius than 3 Å considering the steric exclusion of solute at the channel walls. So 3 Å is a lower radius limit.¹⁰⁾

Fig. 1. Relationship between the Membrane Permeability Clearance (P_m) and the Free Diffusion Coefficient (D)

Key: Inulin (●), PEG 900 (○), Mannitol (■), Erythritol (□), Glycerol (▲), Thiourea (Δ), Urea (★). Values represent mean $\pm$ S.E. of more than five experiments. The regression line at the lower diffusion coefficients is $y = 1.07 \cdot 10^{-4}x + 0.0862$ ($r = 0.992$, $p < 0.01$).

The contribution of the transcellular permeation was examined by the efflux experiment for urea with the highest P_m value (Fig. 2). The semi-logarithmic plot of the efflux rate versus time forms a straight line and the slope gives the first-order rate constant (K) (min^{-1}). The efflux clearance (P_e) was estimated as the product of K and the intracellular volume (V) (μl). P_e is the sum of apical (P_a) and basolateral membrane clearance (P_b). If the ratio of P_a to P_e is α , that of P_b to P_e is $(1 - \alpha)$. The transcellular permeation clearance (P_t) from the apical to basolateral sides, thus, is shown by P_a and P_b :

$$1/P_t = (1/P_a) + (1/P_b) \quad \text{Eq. 1}$$

Using Eq. 1, P_t can also be described by P_e and α :

$$P_t = \alpha(1 - \alpha) \cdot P_e = \alpha(1 - \alpha) \cdot K \cdot V \quad \text{Eq. 2}$$

Although it is difficult to estimate α , $\alpha(1 - \alpha)$ cannot exceed 0.25 by algebraic definition and thus, it is possible to obtain an upper limit to the magnitude of P_t . This derivation ignores the effects of unstirred water layers since those unstirred water layers that decrease cellular efflux and those that decrease transcellular permeation are effectively identical.

Fig. 2. Efflux Rate of ^{14}C -Urea from Colonic Segments Preloaded with the Tracers for 2 h

Values represent mean $\pm$ S.E. of three experiments.

In Table II, the residual value for urea, which was obtained by subtracting the extrapolated values on the straight line from P_m , Fig. 1, and its uncorrected value for the unstirred water layer are shown as P_m' and $P_{m,app}'$, respectively. K , V and an upper limit of P_t obtained by Eq. 2 are also listed in Table II. The mean of P_t was less than about 50% of the $P_{m,app}'$ (Table II). This indicates that the urea permeation, which was higher than expected from the linearity in Fig. 1, occurs essentially via the paracellular route, but the contribution of the transcel-

lular permeation to the urea flux cannot be ruled out.

In summary, there are at least two pores in the colonic membrane of the rat. One is the large pore unrestricted to diffusive flow and the other is the restricted pore with a radius of 3 Å as a lower limit. Also the transcellular route may contribute to the transepithelial flux of the smaller nonelectrolyte.

TABLE II. Residual P_m (P_m'), $P_{m,app}'$, Efflux Rate Constant (K), Intracellular Volume (V) and Maximal Transcellular Permeation Clearance (P_t) for Urea

P_m , a)	$P_{m,app}'$, b)	K c)	V d)	P_t e)	
($\mu\text{l}/\text{min}/\text{cm}^2$)		(min^{-1})	($\mu\text{l}/\text{cm}^2$)	($\mu\text{l}/\text{min}/\text{cm}^2$)	
0.680 ± 0.033	0.477 ± 0.020	0.0269 ± 0.0006	35.3 ± 2.2	0.237 ± 0.015	(mean $\pm$ S.E.)

a) Residual values obtained by subtracting the extrapolated values on the straight line from P_m in Fig. 1 ($n \geq 5$).

b) P_m' uncorrected for the unstirred water layer ($n \geq 5$).

c) From Fig. 2 ($n = 3$).

d) Difference of tissue segment volume and extracellular volume ($n = 8$).

e) An upper limit obtained by Eq. 2.

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(Received August 1, 1989)