Characterization of Drug Transport Through Tight-Junctional Pathway in Caco-2 Monolayer: Comparison with Isolated Rat Jejunum and Colon

Yoshihiro Tanaka,[1] Yoko Taki,[1] Toshiyasu Sakane,[1] Tanekazu Nadai,[1] Hitoshi Sezaki,[1] and Shinji Yamashita[1,2]

Received August 24, 1994; accepted October 27, 1994

Drug transport through the tight-junctional pathway in Caco-2 monolayer was studied by examining the relationship between its permeability to hydrophilic drugs and membrane conductance. Compared with the rat isolated jejunum or colon, Caco-2 monolayer displayed high electrical resistance and low conductance, as well as low permeability to sulfanilic acid and FITC-dextran (M.W. 4000). However, there was a linear relationship between the drug permeability and partial Cl^- ion conductance for Caco-2 monolayer, rat jejunum and colon. Hence, the permeability to those drugs per unit of Cl^- conductance is similar in the three membranes, suggesting that the size (radius) of the tight-junctional pathway in the three membranes is similar. In addition, when the electrical resistance of Caco-2 monolayer was reduced to the same level as that of the jejunum or colon by pretreatment with disodium ethylenediaminetetraacetate, its permeability to FITC-dextran became significantly higher than that of other membranes. Accordingly, the high resistance and the low permeability of Caco-2 monolayer compared with rat intestinal membrane may be due to structural differences between the membranes, rather than a difference in the tightness of the junction.

KEY WORDS: intestinal transport; rat jejunum; rat ileum; Caco-2 cells; FITC-dextran.

INTRODUCTION

Two possible pathways exist for drug permeation in the intestinal membrane, the transcellular and the paracellular route (1,2). Generally, the paracellular route refers to the tight-junctional pathway between cells and can be regarded as a pore filled with water. This route is considered to be significant for the permeation of hydrophilic drugs, whereas lipophilic drugs permeate mainly through the transcellular route. The electrical resistance of the membrane (Rm) is one important indicator of the permeability of this route, since this parameter represents the resistivity of the tight-junction against the diffusion of ions (3). By using an *in vitro* electrophysiological technique, we have revealed that sulfanilic acid (SA), a low lipophilic drug due to the complete ionization at the neutral pH, permeates the rat jejunal membrane mainly via the paracellular route (4). Also, disodium ethylenediaminetetraacetate (EDTA), a powerful chelating agent, selectively enhanced the permeability of the paracellular route to drugs (5).

Caco-2 monolayers may serve as a model for predicting oral drug absorption (6–8). Although Caco-2 cells originated from the human colon carcinoma, they acquire many features of absorptive intestinal cells during culture, such as the microvillous structure and carrier mediated transport systems for sugars, amino acids and several drugs (6,9–12). These features of Caco-2 cells are similar to those of small intestinal, rather than colonic cells. Also, adjacent cells adhere through tight-junctions formed at the apical side of the monolayer. The formation of the tight-junction is usually confirmed by measuring the Rm. Since the Rm of Caco-2 monolayer is higher than that of the small intestinal membrane (9,13,14), it has been considered that Caco-2 cells adhere more tightly to each other than the small intestinal cells. This might explain the low permeability of this membrane to the hydrophilic drugs that permeate the intestinal membrane mainly through the tight-junctional route.

In this study, the tight-junctional permeability of Caco-2 monolayer was investigated by using an *in vitro* electrophysiological technique. The permeability to SA and FITC-dextran (DEX, M.W. 4000) and the electrical conductance of Caco-2 monolayer were determined and compared with those of the isolated rat jejunum and colon.

MATERIALS AND METHODS

Materials

The Caco-2 cell line was obtained from American Type Culture Collection (Rockville, Md.) at passage 17. Dulbecco's modified Eagle medium (DMEM), non-essential amino acids (NEAA), fetal bovine serum (FBS), L-glutamate, trypsin (0.25%)-EDTA (1 mM) and antibiotic-antimycotic mixture (10000 U/ml penicillin G, 10000 μg/ml streptomycin sulfate and 25 μg/ml amphotericin B in 0.85% saline) were purchased from Gibco Laboratories (Lenexa, KS). DEX was purchased from Sigma Chemical Co., (St. Louis, MO). All other reagents were of the highest purity.

Preparation of Caco-2 Monolayer

Caco-2 cells were grown in DMEM supplemented with 10% FBS, 1% L-glutamate, 1% NEAA and 5% antibiotic-antimycotic solution at 37°C in culture flasks (Nippon Becton Dickinson Co., Ltd., Tokyo Japan) in a humidified air-5% CO_2 atmosphere. Cells were harvested with trypsin-EDTA and seeded on polycarbonate filters (0.3 μm pores, 4.71 cm^2 growth area) inside Transwell cell culture chambers (Costar, Cambridge, MA) at a density of 3×10^5 cells/filter. The culture medium (1.5 ml in the insert and 2.6 ml in the well) was replaced every 48 h for the first 6 days and every 24 h thereafter (11). After 15–18 days in culture, the filter with Caco-2 monolayer was removed from the well and mounted in Ussing type chambers for the following experiments (15).

Preparation of the Isolated Sheet of Rat Jejunum and Colon

The lower jejunum or colon (about 5 cm) was removed from Wistar strain male rats weighing 200–250 g, and opened along the mesenterium to give a flat sheet. After washing the intestinal contents with ice-cold transport medium (see be-

[1] Faculty of Pharmaceutical Sciences, Setsunan University, Nagaotoge-cho, Hirakata, Osaka 573-01, Japan

[2] To whom correspondence should be addressed.

0724-8741/95/0400-0523$07.50/0 © 1995 Plenum Publishing Corporation

low), the muscle layer of the membrane was stripped using a microscope slide glass and forceps (16). The mucosal sheet which consisted of the epithelial layer and underlying connective tissue was immediately mounted in Ussing type chambers.

Measurement of the Permeability and the Electrical Resistance of the Membrane

Both sides of the membrane in the chamber were filled with 11 ml of transport medium and stirred by bubbling with 95% CO_2–5% O_2 mixed gas. The transport medium was Hank's balanced salts solution (HBSS) supplemented with 25 mM glucose. The buffer pH was adjusted to 7.0 using HEPES. The electrical membrane resistance (Rm) was calculated from the change in the transmembrane potential difference caused by the external constant current across the membrane, taking into account the resistance of the bathing solutions. The equipment used for electrical measurements was same as that described previously (17). After a 25 min incubation of the membrane with transport medium, sulfanilic acid (SA, 10 mM) or FITC-dextran (DEX, 2 mM) was introduced into the mucosal side. Thereafter, aliquots of samples were taken from the serosal side every 10 min for 1 hr. The volume of the serosal solution was maintained by adding fresh transport medium. The mucosal-to-serosal permeability of SA or DEX (P_{SA}, P_{DEX}) was calculated from its flux rate estimated as the rate of increase in the serosal concentration. These experiments were performed under temperature controlled condition at 37°C.

The effect of disodium ethylenediaminetetraacetate (EDTA) on Rm and P_{DEX} was estimated by pretreating the mucosal side of the membrane with 10 mM EDTA solution for 30 min before introducing DEX.

Estimation of Relative Permeability of Ions

The permeability ratios among Na^+, K^+ and Cl^- (expressed as P_{Cl}/P_{Na} and P_K/P_{Na}) were calculated from two kinds of diffusion potentials, i.e., the NaCl 1/2 dilution potential and Na^+/K^+ biionic potential across the membrane measured by the same method as described previously (17). This experiment proceeded at 20°C to prevent the effect of the active transport of ions.

Calculation of the Partial Conductance of Cl^- (G_{Cl})

Assuming that most of the membrane conductance (the reciprocal of Rm) was caused by the permeation of Na^+, K^+ and Cl^- ions across the membrane in this experiment, partial conductance of Cl^- ion was calculated according to the equations as,

$$G_t = \sum G_i \approx G_{Na} + G_K + G_{Cl} \quad (1)$$

$$G_{Cl} = G_t \times \frac{G_{Cl}}{G_t} = G_t \times \frac{G_{Cl}}{G_{Na} + G_K + G_{Cl}} \quad (2)$$

$$G_i = P_i \cdot C_i \cdot Z_i^2 \cdot F^2/RT \quad (3)$$

$$G_{Cl} = G_t \times \frac{P_{Cl} \cdot C_{Cl}}{P_{Na} \cdot C_{Na} + P_K \cdot C_K + P_{Cl} \cdot C_{Cl}} \quad (4)$$

where G_t is the total conductance obtained as the reciprocal of Rm, and G_i, P_i and C_i represent the partial conductance, the relative permeability and the concentration of ion i, respectively. Z, F, R and T have their usual meanings. In the following study, G_t was calculated from the average Rm value during the last 30 min (50–80 min in Fig. 1) in each experiment.

Analytical Methods

The concentration of SA was estimated spectrophotometrically as described by Kimura *et al.* (18). DEX was detected fluorometrically by means of high performance liquid chromatography (LC-6A Shimadzu Co., Kyoto, Japan) with a reversed phase column (Inertosil ODS-2, Gaskurokogyo, Tokyo, Japan) equipped with a fluorescence spectro-monitor (RP-530, Shimadzu Co.). The analytical conditions for DEX were as follows: mobile phase: pH 7.4 phosphate buffer, flow rate: 1.0 ml/min, wave length: 495 nm for excitation and 514 nm for emission, column temperature: 40°C.

RESULTS

The time-course of Rm in Caco-2 monolayer, isolated rat jejunum and colon obtained at 37°C is shown in Figure 1. The Rm of three membranes became stable after a 30 min incubation. The average Rm value during last 30 min (50–80 min) was 415.6, 87.4 and 41.8 $\Omega \cdot cm^2$ for Caco-2 monolayer, the colon and the jejunum, respectively. These values are compatible with the Rm of each membrane reported previously (3,11).

The permeability ratios of Cl^- and K^+ to Na^+ ions in the three membranes were calculated from the diffusion potentials and are summarized in Table 1 along with the free mobility ratios of these ions in aqueous solution (19). In the jejunum, the relative permeability of Cl^- ion was low compared with other two membranes and about half of the free mobility ratio. The relative permeability of K^+ was close to the free mobility ratio in all membranes. It is obvious that the permeability of these membranes to anion was relatively low compared with that to cation, especially in the jejunum. Based on these data, the G_{Cl} in three membranes was cal-

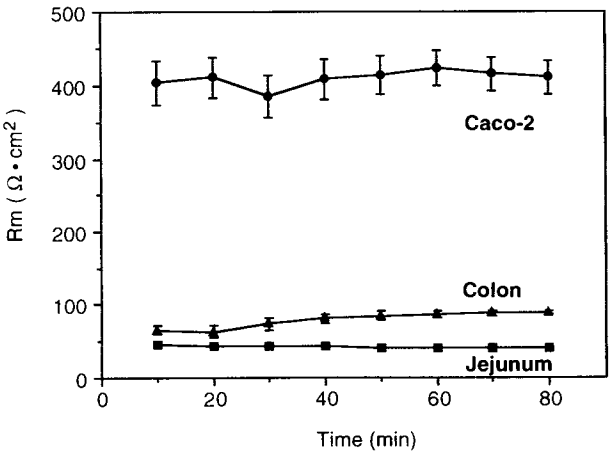

Fig. 1. The time course of the Rm of the isolated rat jejunum, colon and Caco-2 monolayer. Each point represents the mean ± SE of at least eight experiments.

Table 1. The Ion-Selective Permeability of Three Membranes

Membrane	P_{Cl}	P_{Na}	P_K
Jejunum	0.75	1	1.49
Colon	1.15	1	1.49
Caco-2	1.02	1	1.45
Free mobility[a]	1.61	1	1.54

Results are expressed as the ratio to the permeability of the Na^+ ion.

[a] The free mobility ratio in an aqueous solution of Cl^- or K^+ relative to that of Na^+ (from Ref. 14).

culated. The ratio of G_{Cl} to G_t was 0.42, 0.52 and 0.49 in the jejunum, the colon and Caco-2 monolayer, respectively.

Figure 2 shows the time course of mucosal to serosal transport of SA and DEX across the three membranes. The permeability of both drugs, P_{SA} and P_{DEX} calculated from the slope of the linear portion in each line, was the highest in the jejunum and lowest in Caco-2 monolayer. In Figure 3, P_{SA} and P_{DEX} correlated with the G_{Cl} of each membrane. There was a good linear relationship among the three membranes, suggesting that the permeability of SA or DEX per unit of Cl^- conductance is similar in all membranes.

The P_{DEX} and Rm after the pretreatment with 10 mM EDTA were summarized in Table 2. EDTA enhanced P_{DEX} with reducing Rm in all three membranes. In Caco-2 monolayer, P_{DEX} and Rm were dramatically changed by EDTA compared with those in other membranes, showing that Caco-2 monolayer is the most sensitive to EDTA treatment among the three membranes. Also, EDTA reduced the Rm of the three membranes to the same level, whereas P_{DEX} was highest in Caco-2 monolayer.

DISCUSSION

We had already demonstrated that SA permeates the rat jejunum mainly via the paracellular route (4). Also, the permeabilities of each membrane to SA and DEX were compatible with those obtained for the typical paracellular permeants of similar molecular weight, such as mannitol or polyethylene glycol (14,20). In this study, the permeability of Caco-2 monolayer to SA and DEX was lower than that of the jejunum or the colon, reflecting the high Rm value in Caco-2 monolayer. Therefore, cells in Caco-2 monolayer appear to adhere to each other more tightly than those in the intestinal membrane. However, the correlation of drug permeability with partial conductance of Cl^- of each membrane indicates another interpretation of these data. The theoretical handling is as follows. For substance i which permeates the membrane via a pore existing within the membrane, Pappenheimer (21) defined the membrane permeability (Pi) as,

$$P_i = \frac{D_i \cdot A_i}{\Delta X} \tag{5}$$

where D_i is the diffusivity of a substance i in aqueous solution and ΔX is the membrane thickness. A_i is the permeability factor which mediates the permeation of substance i through the pore and is defined as,

$$A_i = \frac{Ap}{Am} \cdot F^1{}_i \cdot F^2{}_i \tag{6}$$

where Ap/Am expresses the ratio of the total area of the pore (Ap) to the total membrane surface area (Am). F^1i and F^2i are the factors concerning the steric hindrance that occurs when the substance enters the pore (F^1i) and when it passes within the pore (F^2i). Both F^1i and F^2i are functions of the ratio of the molecular radius of substance i to the pore radius. If F equals one, the substance can permeate the pore without any steric hindrance and, if F equals zero, no permeation will occur. Another factor which restricts the permeation through the pore is electrical repulsion by the wall of the pore. As shown in Table 1, all three membranes, especially the jejunum, showed cation selective permeability because the wall of pore is considered to be lined with dipolar groups, such as carbonyl groups, whose negative ends point into the pore (22,23). Therefore G_{Cl}, which is proportional to the permeability of Cl^- ion (P_{Cl}), was calculated

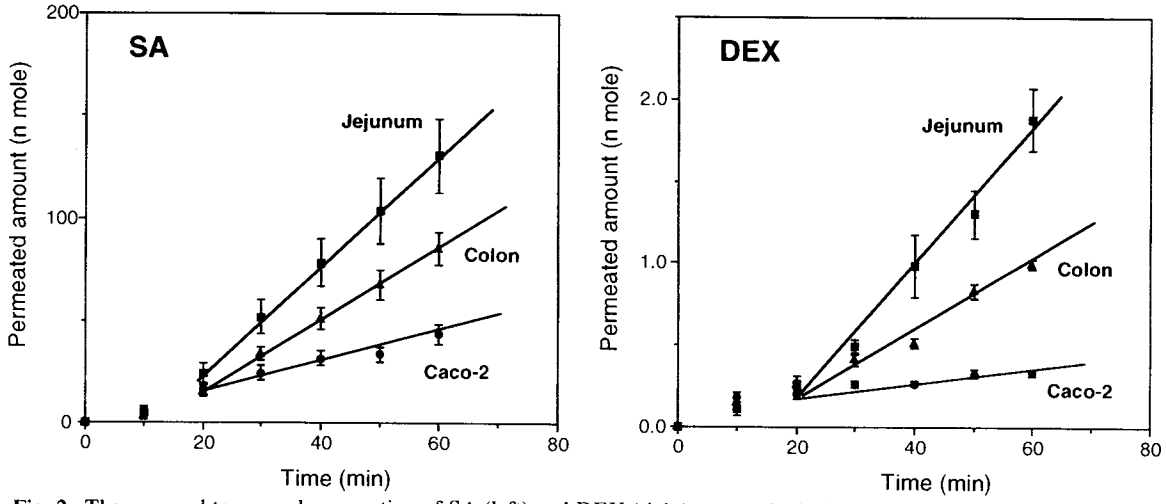

Fig. 2. The mucosal to serosal permeation of SA (left) and DEX (right) across the isolated rat jejunum, colon and Caco-2 monolayer. The initial concentration of drugs introduced to the mucosal side was 10 mM for SA and 2 mM for DEX. Each point represents the mean ± SE of at least four experiments.

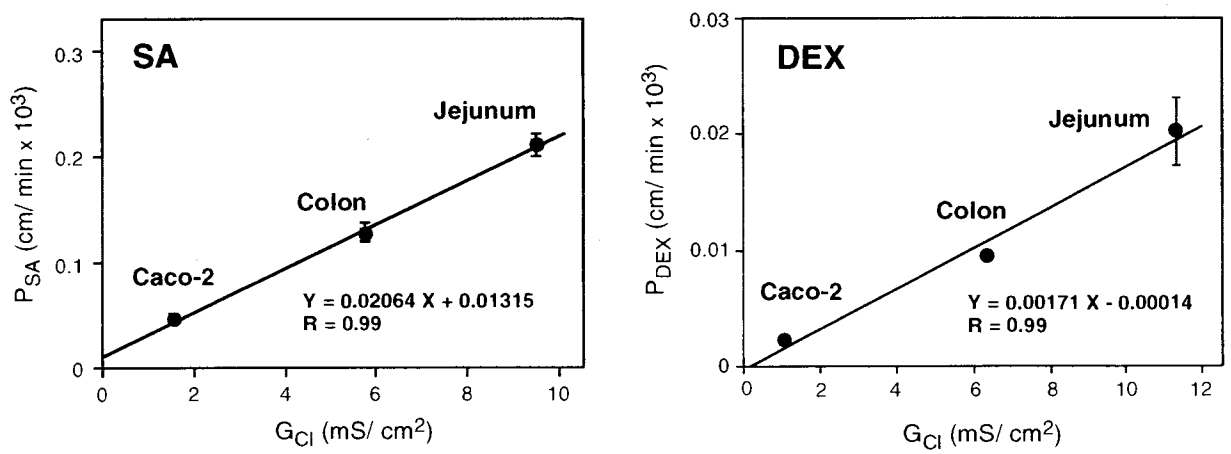

Fig. 3. The relationship between the partial conductance of Cl^- and the permeability to SA (left) and DEX (right) among the isolated rat jejunum, colon and Caco-2 monolayer. Each point represents the mean ± SE of at least four experiments.

and compared with the permeation of the negatively charged drugs, SA and DEX. G_{Cl} is expressed as,

$$G_{Cl} = P_{Cl} \cdot C_{Cl} \cdot Z_{Cl}^2 \cdot F^2/RT$$
$$= D_{Cl} \cdot A_{Cl} \cdot C_{Cl} \cdot Z_{Cl}^2 \cdot F^2/\Delta X \cdot RT \quad (7)$$

Using equations (5) and (7), P_i/G_{Cl}, which corresponds to the slope in Figure 3 was calculated as,

$$\frac{P_i}{G_{Cl}} = \frac{D_i}{D_{Cl} \cdot C_{Cl}} \cdot \frac{A_i}{A_{Cl}} \cdot RT/F^2 \quad (8)$$

In equation (8), since the diffusivity of each substance (D_i, D_{Cl}) and the concentration of Cl^- (C_{Cl}) should be the same in all experiments and RT/F^2 is constant, P_i/G_{Cl} can be regarded as a function of A_i/A_{Cl},

$$P_i/G_{Cl} \propto A_i/A_{Cl} = \frac{(Ap/Am) \cdot F_i^1 \cdot F_i^2}{(Ap/Am) \cdot F_{Cl}{}^1 \cdot F_{Cl}{}^2} = \frac{F_i{}^1 \cdot F_i{}^2}{F_{Cl}{}^1 \cdot F_{Cl}{}^2} \quad (9)$$

P_{SA}/G_{Cl} and P_{DEX}/G_{Cl} in the three membranes were calculated in Table 3. If a pore in Caco-2 monolayer, the tight-junctional pathway, is narrower than that in other membranes, the permeation of large molecules, such as DEX, should be highly restricted by severe hindrance compared with the permeation of Cl^-. Thus the ratios in Table 3 should become lower in Caco-2 monolayer than in the jejunum or the colon. However, the ratio is highest in Caco-2 monolayer and lowest in the colon for both drugs, although there are no significant differences. Assuming that Cl^- is small enough to escape serious steric hindrance in all membranes, the results shown in Figure 3 and Table 3 suggest that the permeation of SA or DEX is restricted to a similar level in all membranes. Since the factors of the hindrance ($F_{SA}{}^1 \cdot F_{SA}{}^2$ or $F_{DEX}{}^1 \cdot F_{DEX}{}^2$) are functions of the pore radius, this result means that the effective pore radius is essentially the same in the three membranes.

If the differences in Rm and P_{SA} or P_{DEX} among the three membranes are not due to the difference in the tightness of the junctional pathway, they can be explained by a structural difference of the membrane. As shown in Figure 4, the jejunal membrane has a specific structure, villous structure, that increases the effective surface area for absorption, whereas Caco-2 monolayer is flatter. The permeability to drugs or Rm, which was calculated based on the unit area of the membrane, might be different because of the difference of the effective area. Since the number of tight-junctions per unit area is larger in the jejunum than in Caco-2 monolayer, it is reasonable to consider that the high Rm and the low permeability to hydrophilic drugs in Caco-2 monolayer are due to the small number of junctions per unit area, rather than a difference in their tightness. In the case of colon, the presence of some folds in the membrane might increase the effective surface area compared with Caco-2 monolayer. Although there are no significant differences, both P_{SA}/G_{Cl} and P_{DEX}/G_{Cl} values were the lowest in the colon in Table 3. Thus, the junction in the colon may be slightly tighter than that in the jejunum or Caco-2 monolayer. Artursson *et al.* (14) have investigated the molecular weight-dependent per-

Table 2. The Effect of EDTA on the Rm and P_{DEX} of Three Membranes

	Control		Pretreatment with EDTA	
	Rm ($\Omega \cdot cm^2$)	P_{DEX} (cm/min × 10^5)	Rm ($\Omega \cdot cm^2$)	P_{DEX} (cm/min × 10^5)
Jejunum	37.4 ± 1.9	2.02 ± 0.25	23.9 ± 1.5	4.87 ± 0.32
Colon	82.6 ± 3.1	0.948 ± 0.025	33.3 ± 1.1	3.33 ± 0.47
Caco-2	467.9 ± 14.1	0.215 ± 0.024	30.4 ± 1.6	7.55 ± 0.20

Results are expressed as the means ± SE of at least four experiments.

Table 3. The Ratio of Drug Permeability to Cl^- Conductance in Three Membranes

	P_{SA}/G_{Cl}[a]	P_{DEX}/G_{Cl}[a]
Jejunum	0.0314 ± 0.0042	0.00176 ± 0.00018
Colon	0.0294 ± 0.0010	0.00150 ± 0.00006
Caco-2	0.0467 ± 0.0071	0.00206 ± 0.00024

The results are expressed as the means ± SE of at least four experiments.

[a] Unit of the ratio is (cm/min)/(S/cm^2) = (cm^3)/(S · min).

meability of the rat ileum, colon and Caco-2 monolayer to hydrophilic markers *in vitro* and concluded that those membranes have similar permeability profiles to those of human intestine *in vivo*. Our theory is further supported by their findings.

EDTA is a well known enhancer for intestinal drug absorption and is reported to augment the tight-junctional permeability by means of its chelating activity (24,25). Therefore, the enhanced P_{DEX} and reduced Rm detected here can be assumed to represent a change in tight-junctional permeability. After exposure to EDTA, P_{DEX} in Caco-2 monolayer was significantly higher than that in the jejunum or colon despite the similar Rm in all membranes. This suggests that the steric hindrance for DEX permeation is small in Caco-2 monolayer because its tight-junction became looser than that of other membranes. However, due to the lesser numbers of junctional pathways, Rm in Caco-2 monolayer was the same as that in other membranes.

In conclusion, we present a new interpretation of the tight junctional permeation of drugs in Caco-2 monolayer based upon the conductance analysis. Although more detailed investigations are necessary to completely characterize the drug permeation across Caco-2 monolayer, the results presented here are significant when estimating oral drug absorption from *in vitro* study using Caco-2 monolayer.

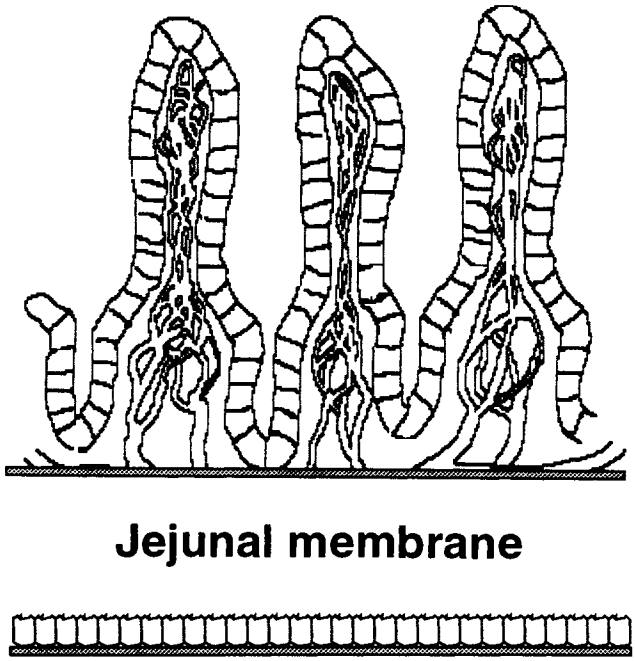

Fig. 4. A schematic representation of membrane structures.

REFERENCES

1. M. J. Jackson, C. Y. Tai and J. E. Steane. Weak electrolyte permeation in alimentary epithelia. *Am. J. Physiol.* 240, G191–G198 (1981).
2. G. Barnett, S. Hui and L. Z. Benet. Effects of theophylline on salicylate transport in isolated rat jejunum. *Biochim. Biophys. Acta.* 507, 517–528 (1978).
3. D. W. Powell. Barrier function of epithelia. *Am. J. Physiol.* 241, G275–288 (1981).
4. S. Yamashita, M. Takahashi, M. Masada, T. Nadai, A. Kobayashi, Y. Kurosaki, T. Nakayama and T. Kimura. Effect of experimental acute renal failure on barriers to permeation of a polar drug in rat jejunum: an electrophysiological study. *Chem. Pharm. Bull.* 36, 2538–2543 (1988).
5. S. Yamashita, H. Saitoh, K. Nakanishi, M. Masada, T. Nadai and T. Kimura. Effect of diclofenac sodium and disodium ethylenediaminetetraacetate on electrical parameters of the mucosal membrane and their relation to the permeability enhancing effects in the rat jejunum. *J. Pharm. Pharmacol.* 39, 621–626 (1986).
6. A. R. Hilgers, R. A. Conradi and P. S. Burton. Caco-2 cell monolayers as a model for drug transport across the intestinal mucosa. *Pharm. Res.* 7, 902–910 (1990).
7. G. Wilson, I. F. Hassan, C. J. Dix, I. Williamson, R. Shah, M. Mackay and P. Artursson. Transport and permeability properties of human Caco-2 cells: an in vitro model of the intestinal epithelial cell barrier. *J. Controlled Release* 11, 25–40 (1990).
8. P. Artursson and J. Karlsson. Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (Caco-2) cells. *Biochem. Biophys. Res. Commun.* 175, 880–885 (1991).
9. I. J. Hidalgo, T. J. Raub and R. T. Borchardt. Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. *Gastroenterology* 96, 736–749 (1989).
10. A. Blais, P. Bissonnette and A. Berteloot. Common Characteristics for Na^+-dependent sugar transport in Caco-2 cells and human fetal colon. *J. Membrane Biol.* 99, 113–125 (1987).
11. I. J. Hidalgo and R. T. Borchardt. Transport of a large neutral amino acid (phenylalanine) in a human intestinal epithelial cell line: Caco-2. *Biochim. Biophys. Acta.* 1028, 25–30 (1990).
12. A. H. Dantzig and L. Bergin. Uptake of the cephalosporin, cephalexin, by a dipeptide transport carrier in the human intestinal cell line, Caco-2. *Biochim. Biophys. Acta.* 1027, 211–217 (1990).
13. P. Artursson. Epithelial transport of drugs in cell culture. I: A model for studying the passive diffusion of drugs over intestinal absorbtive (Caco-2) *Cells. J. Pharm. Sci.* 79, 476–482 (1990).
14. P. Artursson, A. L. Ungell and J. E. Lofroth. Selective paracellular permeability in two models of intestinal absorption: Cultured monolayers of human intestinal epithelial cells and rat intestinal segments. *Pharm. Res.* 8, 1123–1129 (1993).
15. T. Kimura, S. Yamashita, K. S. Kim and H. Sezaki. Electrophysiological approach to the action of taurine on rat gastric mucosa. *J. Pharm. Dyn.* 5, 495–500 (1982).
16. H. N. Nellans, R. A. Frizzell and S. G. Schultz. Brush-border processes and transepithelial Na and Cl transport by rabbit ileum. *Am. J. Physiol.* 226, 1131–1141 (1974).
17. S. Yamashita, M. Masada, T. Nadai and T. Kimura. Effect of adjuvants on charge-selective permeability and electrical resistance if rat jejunal membrane. *J. Pharm. Sci.* 79, 579–583 (1990).
18. T. Kimura, K. S. Kim and H. Sezaki. Effect of taurine on drug absorption from the rat gastrointestinal tract. *J. Pharmacobio Dyn.* 4, 35–41 (1981).
19. J. H. Moreno and J. M. Diamond. Discrimination of monova-

lent inorganic cations by "Tight" junctions of gallbladder epithelium. *J. Membrane Biol.* 15, 277–318 (1974).
20. M. Tomita, M. Hayashi and S. Awazu. Comparison of absorption-enhancing effect between sodium caprate and disodium ethylenediaminetetraacetate in Caco-2 cells. *Biol. Pharm. Bull.* 17, 753–755 (1994).
21. J. R. Pappenheimer. Passage of molecule through capillary walls. *Physiol. Rev.* 33, 387–423 (1953).
22. P. G. Ruifrok and W. E. Mol. Paracellular transport of inorganic and organic ions across the rat ileum. *Biochem. Pharmacol.* 32, 637–640 (1983).
23. P. H. Barry and J. M. Diamond. A theory of ion permeation through membranes with fixed neutral sites. *J. Membrane Biol.* 4, 295–330 (1971).
24. M. M. Cassidy and C. S. Tidball. Cellular mechanism of intestinal permeability alterations produced by chelation depletion. *J. Cell Biol.* 32, 685–697 (1967).
25. T. Murakami, N. Yata, H. Tamauchi and H. Kamada. Studies of absorption promoters for rectal delivery preparations. II. A possible mechanisms of promoting efficacy of enamine derivatives in rectal absorption. *Chem. Pharm. Bull.* 30, 659–665 (1982).