Selective Paracellular Permeability in Two Models of Intestinal Absorption: Cultured Monolayers of Human Intestinal Epithelial Cells and Rat Intestinal Segments

Per Artursson,[1] Anna-Lena Ungell,[2,3] and Jan-Erik Löfroth[2]

Received August 10, 1992; accepted January 21, 1993

New data on the permeabilities of hydrophilic markers in two commonly used *in vitro* models, i.e., excised intestinal segments from the rat and monolayers of Caco-2 cells, are presented. The results are compared to human *in vivo* data. Two groups of hydrophilic marker molecules were tested: (1) monodisperse polyethylene glycols of molecular weights ranging from 194 to 502 g/mol and (2) a heterogeneous group of molecules consisting of urea, creatinine, erythritol, and mannitol (60–182 g/mol). The permeabilities of the marker molecules showed a nonlinear dependence on the molecular weight and decreased in the order rat ileum > rat colon > Caco-2 cells. Surprisingly, the polyethylene glycols permeated more easily than the other marker molecules, indicating that characteristics other than molecular weight, e.g., the flexibility of the structure, may also be important for permeation through the membrane. Comparisons with the published permeability profiles of polyethylene glycols in human intestinal segments *in vivo* (i.e., calculated permeability coefficients as a function of molecular weight) indicate that the human intestine is more permeable than the *in vitro* models. However, the permeability profiles of the corresponding segments in the human intestine and the *in vitro* models were comparable. Thus, good correlations were established between permeabilities of the human ileum and rat ileum and between those of human colon, rat colon, and the Caco-2 cells. We conclude that the paracellular absorption in humans can be studied mechanistically in these *in vitro* models.

KEY WORDS: paracellular permeability; drug absorption models; hydrophilic markers; Caco-2 cells; rat intestinal segments.

INTRODUCTION

A major function of the intestinal epithelium is to absorb nutrients, electrolytes, and water. At the same time, the epithelium should serve as a barrier to potentially harmful compounds and microorganisms in the intestinal lumen. This selective barrier function can be characterized by certain hydrophilic molecules, i.e., those that permeate the epithelium by passive diffusion. Usually, metabolically inert molecules of different molecular weights are used. These include creatinine, mannitol, lactulose, rhamnose, raffinose, inulin, ^{51}Cr-EDTA, and polydisperse polyethylene glycols with molecular weights between 200 and 2000 (see Ref. 1). These markers are too hydrophilic to be distributed into the lipophilic cell membranes. Instead, they are absorbed across the intercellular spaces by the paracellular pathway.

The intercellular spaces are gated by tight junctions which regulate the absorption by the paracellular pathway. The barrier function of the tight junctions contributes significantly to the total barrier function of the intestinal epithelium. Indeed, it is generally accepted that variations in tight junction permeability explain differences in barrier function between different epithelia. Measurements of paracellular permeability can therefore be used to characterize the barrier function of the intestinal epithelium.

Most studies on intestinal permeability have been performed after oral intake or after intestinal load of the hydrophilic marker molecules. These studies have shown that the permeability to different marker molecules varies along the intestinal canal. In general, the permeability decreases in the order jejunum > ileum > colon (e.g., Refs. 2–4). The permeability of a given intestinal segment varies with the size of the marker molecule. Smaller molecules can permeate more easily than larger ones. For a homogeneous series of compounds (e.g., polyethylene glycols), the permeability is inversely related to the molecular weight (4). When the permeabilities of compounds with different molecular geometries are compared, other size measures such as cross-sectional diameter have been suggested (5).

Although intestinal permeability has been extensively characterized *in vivo*, few systematic studies have been performed *in vitro*. This is surprising since excised intestinal segments and, more recently, monolayers of intestinal epithelial cells have been extensively used to study the barrier function of normal and affected epithelia to nutrients, ions, and drugs (6–12). Thus, no studies on the influence of molecular weight on the permeation across *in vitro* models have been published. Such information would be valuable in the extrapolation of findings in the *in vitro* models to the *in vivo* situation.

In this study, the permeabilities of two groups of hydrophilic marker compounds were investigated in two commonly used *in vitro* models: excised intestinal segments (ileum and colon) from the rat and monolayers of an intestinal epithelial cell line (Caco-2), originally derived from a human colorectal carcinoma (13). The first group of hydrophilic compounds was comprised of a series of monodisperse polyethylene glycols (M_w, 194–502 g/mol). The second group was comprised of relatively small molecules (M_w, 60–182 g/mol) with varying molecular geometry. Permeability profiles were established and compared with permeability profiles of the polyethylene glycols in human jejunum, ileum, and colon calculated from the literature (4). The permeability profiles of human ileum and rat ileum were in good agreement, as were those of human colon, rat colon, and Caco-2 cells.

MATERIALS AND METHODS

Solutions

Tracer amounts of the following radiolabeled com-

[1] Department of Pharmaceutics, University of Uppsala, Box 580, S-752 23 Uppsala, Sweden.
[2] Drug Delivery Research, Astra Hässle AB, S-431 83 Mölndal, Sweden.
[3] To whom correspondence should be addressed.

0724-8741/93/0800-1123$07.00/0

pounds were added to a Krebs–bicarbonate Ringer's solution (KBR; excised segments) and to Hank's balanced salt solution (HBSS; cell monolayers): ^{14}C-urea (American Radiolabeled Chemicals Inc.), ^{14}C-creatinine (American Radiolabeled Chemicals Inc.), ^{14}C-erythritol (American Radiolabeled Chemicals Inc.), ^{14}C-mannitol (New England Nuclear), and monodisperse ^{14}C-PEG (polyethylene glycol). ^{14}C-PEG (400) was obtained from Berol Kemi Stenungsund AB, Sweden, and was size fractioned into the molecular fractions 194, 238, 282, 326, 370, 414, 458, and 502 g/mol, as described by Johansson *et al.* (22).

The KBR solution had the following composition (m*M*): 108.0 NaCl, 4.7 KCl, 1.8 Na_2HPO_4, 0.4 KH_2PO_4, 15.0 $NaHCO_3$, 1.2 $MgSO_4$, 1.25 $CaCl_2$, 11.5 D-glucose, and the additional respiratory substrates 4.9 Na–L-glutamate, 5.4 Na_2–fumarate, and 4.9 Na–pyruvate. It was gassed with a O_2/CO_2 (95/5%) gas mixture to a constant pH of 7.4.

The HBSS solution consisted of Hank's balanced salt solution (pH 7.4) and contained benzylpenicillin (100 U/mL), streptomycin (10 μg/mL), and Hepes (25 m*M*). All monolayer experiments were carried out in air at 37°C and 95% relative humidity.

Preparation

Excised Segments. Female Sprague–Dawley rats weighing 250–300 g were used in this study and were received at least 1 week prior to use. The rats had free access to food and water prior to sacrifice.

After sacrifice with CO_2, the whole intestine was removed and washed with cold Krebs–bicarbonate Ringer's solution (KBR) put in a beaker with KBR on ice which was continuously gassed with a O_2/CO_2 (95/5%) gas mixture.

For the experiments, the distal part of the ileum 5 cm proximal to the ileocecal junction and the descending colon were used and were opened along the mesenteric border. Care was taken to avoid the Peyer's patches.

Blunt dissection was used to remove the serosa from the ileum segments and the serosa and muscularis externa from the colon segments. During the preparation the tissues were submerged in KBR (12°C) which was gassed continuously. Each segment was then mounted on a segment holder, which was connected to a transport chamber (modified Ussing chamber) consisting of two 10-mL chambers with rotors for effective stirring of the test solutions as described earlier (8).

Cell Culture. Caco-2 cells, referred to as Caco-2 parent cells (Caco-2p), originating from a human colorectal carcinoma, were obtained from the American Cell Culture Collection, Rockville, MD. Caco-2 cell clone 40 (Caco-2cl 40) was obtained from Dr. Martin Mackay, Ciba-Geigy Pharmaceuticals, Horsham, England (15).

The cells were cultivated on polycarbonate filter cell culture inserts with a filter diameter of 25 or 6.5 mm and a mean pore diameter of 0.45 μm (Transwell, Costar, Cambridge, MA) as described elsewhere (11,15). Caco-2p cells of passage numbers 89–99 and Caco-2cl 40 of passage numbers 16–19 were used. The permeability studies were carried out with cells cultivated in the filter chambers for 3–4 weeks. At this time Caco-2p and Caco-2cl 40 have differentiated into monolayers of polarized cells with well-developed apical brush borders and tight junctions (11,15).

Measurement of Electrical Parameters

Excised Segments. The electrical parameters, PD and SCC, were recorded simultaneously during the experiments as described previously (8). Any tissue with values below 30 or 60 $\Omega \cdot cm^2$ and 3 or 5 mV in PD for ileum and colon, respectively, was omitted before the start of the experiments. Unidirectional flux studies were performed with the tissues unclamped.

Cell Monolayers. The electrical permeabilities of the cell monolayers were determined by electrical resistance measurements using the method of von Bonsdorff *et al.* (14). A direct current of 100 μA was applied across the monolayers and the potential difference was measured. The electrical resistance was then derived from Ohm's law.

Permeability Experiments

Excised Segments. The experiments were started by changing both the mucosal and the serosal KBR solution. The mucosal solution contained the radiolabeled substance to be tested. Samples of 50 μL were withdrawn from the serosal compartment at different time intervals for up to 180 min and were replaced with fresh KBR. Ten milliliters of Ready Safe (Beckman Instruments) was added to each sample and the amount of radioactivity determined in a LKB Rackbeta Scintillation Counter.

Cell Monolayers. HBSS containing trace amounts of the radiolabeled marker molecules was added to the apical side of the cells. The cell monolayers were agitated with a calibrated plate shaker (Titertec, Flow Laboratories Ltd., UK). The agitation minimizes the influence of the aqueous boundary layer on the measured permeability coefficients, as described previously (16).

At five regular time intervals (i.e., when a measurable amount of the marker compound had diffused across the monolayers), the cell culture inserts were moved to another serosal chamber. The radioactive contents in the serosal chambers were analyzed in a liquid scintillation spectrometer (Tricarb 1900 CA, Packard Instruments) and the accumulated radioactivity was calculated for each time point.

Calculations of Permeability Coefficients

In Vitro *Models.* The apparent permeability coefficients (P_{app}) in the *in vitro* models were calculated using Eq. (1).

$$P_{app} = dQ/dt \cdot 1/AC_o \quad (1)$$

where dQ/dt is the steady-state appearance rate of the compound on the serosal side, C_o the initial concentration of the compound on the mucosal side of the membranes, and A the surface area of the membrane exposed to the compound (21).

Calculations of Permeability Coefficients in Humans

Human Data. Apparent permeability coefficients of monodisperse PEGs in the human intestinal segments were calculated from the study by Chadwick *et al.* (4), in which the intestinal absorption of polydisperse PEG was studied *in vivo* in humans by the use of a triple-lumen perfusion technique. The data calculated from the disappearance rates of

the markers from the lumen were used instead of urinary recovery data, since it has been shown that the urinary recovery of polydisperse PEGs is dependent on molecular weight (17). The apparent permeability coefficients were calculated according to Komiya *et al.* (18):

$$P_{app} = \frac{Q}{2\pi rl} \cdot \ln \frac{C_o}{C_l} \quad (2)$$

In this equation Q is the flow rate and C_l/C_o is the fraction of marker molecule remaining in the intestinal lumen of length l and effective radius r. The expression $2\pi rl$ is equal to the inner smooth area of the intestinal segment. The inner smooth areas of the human small intestinal segments were calculated to be approx. 240 cm^2, while the corresponding value for the colon was 336 cm^2 (19).

RESULTS

Transepithelial Electrical Resistance

A common method to characterize the barrier properties of various epithelia in vitro is to measure the transepithelial electrical resistance (TEER), which is a direct measure of the epithelial resistance to passive ion flow (2). A comparison between the TEER of the excised segments and the cell lines is shown in Table I. Rat ileum had a TEER that was approximately three times lower than that of rat colon. The original Caco-2 cell line (Caco-2p) had a TEER that was approximately four times higher than that of rat colon, while a newly isolated clone of Caco-2p (Caco-2cl.40) had a TEER that was significantly higher than that of Caco-2p.

Permeability Profiles

The results from the permeability experiments with the PEGs are shown in Fig. 1. The excised rat ileum was more permeable than both the rat colon and the cell lines. When compared to rat colon the permeabilities of the PEGs in rat ileum were approximately four to six times greater. For both tissues, however, the marked molecular weight dependence of the permeability was not as pronounced for molecular weights above approximately 400.

The permeabilities of the cell lines were more similar to those of rat colon than to rat ileum (Fig. 1). For PEGs of higher molecular weights there was a gradual decrease in the permeability of the cell lines as compared to the rat tissues. Thus, for the largest PEGs the cell lines were approximately 10 and 50 times less permeable than rat colon and ileum, respectively. As for the rat tissues, the molecular weight dependence of the permeability in the cell lines was less pronounced for PEGs with a molecular weight of >400.

The permeability profiles of the second (more heterogeneous) group of markers were different from those of the PEGs (Fig. 2). When represented as a function of molecular weight, the permeabilities of urea, erythritol, creatinine, and mannitol were lower than what could be expected from the results obtained with the PEGs.

A very good correlation between the two cell lines was obtained for the permeabilities of all marker molecules (Fig. 3). Thus, although Caco-2p and Caco-2cl.40 differed in TEER, there was no significant difference in marker permeability between the cell lines (Table I and Fig. 3).

The apparent permeability coefficients for PEG in human jejunum, ileum, and colon were calculated as described above, and the permeability profiles were compared with those obtained in the *in vitro* models (Fig. 4). The human intestinal segments were approximately 10 times more permeable than the corresponding rat segments, and the human jejunum was the most permeable tissue. A linear correlation (r^2 = 0.989) was obtained between the permeability coefficients of human and rat ileum.

Similar but less pronounced differences were observed between the permeability coefficient of human and that of rat colon. In general, the permeability coefficients of human colon were 5–10 times higher than those of rat colon. For larger PEGs (e.g., 502 g/mol) the difference was only twofold. Thus, the permeability of the PEG of the highest molecular weight (502) was more restricted in human segments. This was in agreement with the findings in the human intestinal cell line, Caco-2 (Fig. 4). Although human colon was approximately 20–40 times more permeable than Caco-2 monolayers, the permeability profiles were similar and a linear correlation (r^2 = 0.985) was obtained between the permeability coefficient of human colon and that of Caco-2 cells. The correlations between rat and human colon and rat colon and Caco-2 cells were also acceptable (r^2 = 0.976 and 0.951, respectively).

Table I. Transepithelial Electrical Resistance (TEER) of the Rat Ileum, the Rat Colon, and the Two Cell Lines, Caco-2p and Caco-2cl.40, in the Presented Experiments

Tissue/cell line	Resistance ($\Omega \cdot cm^2$)[a]
Rat ileum	35.3 ± 4.9 (43)
Rat colon	100.6 ± 26.0 (28)
Caco-2p	420.0 ± 26.0 (12)
Caco-2cl.40	662.0 ± 42.0 (12)

[a] Mean values ± SD; number of experiments given in parentheses.

DISCUSSION

In this study the permeabilities of two *in vitro* models, i.e., excised rat intestinal segments and monolayers of an intestinal epithelial cell line, to hydrophilic marker molecules of different molecular weights were investigated. In general, the permeabilities were lower in the *in vitro* models than in the corresponding segments of the human intestine *in vivo*. Several factors that are diminished or absent in the *in vitro* models could contribute to this difference. For instance, the excised intestinal segments are denervated of central nervous control to the enteric plexii of the subepithelial tissue. This situation may affect ion and water transport in the *in vitro* models. Other contributing factors may be the absence of the systemic blood flow, reduced motility, and differences in thickness of the mucus layer. Additional factors that could reduce the permeability further in the cell monolayers are related to the absence of the crypt–villus axis, the low turnover rate of these cells (no signs of cell extrusion), and the absence of goblet cells (20,21).

Despite these differences and the differences in permeability rates, the different permeability profiles in this study

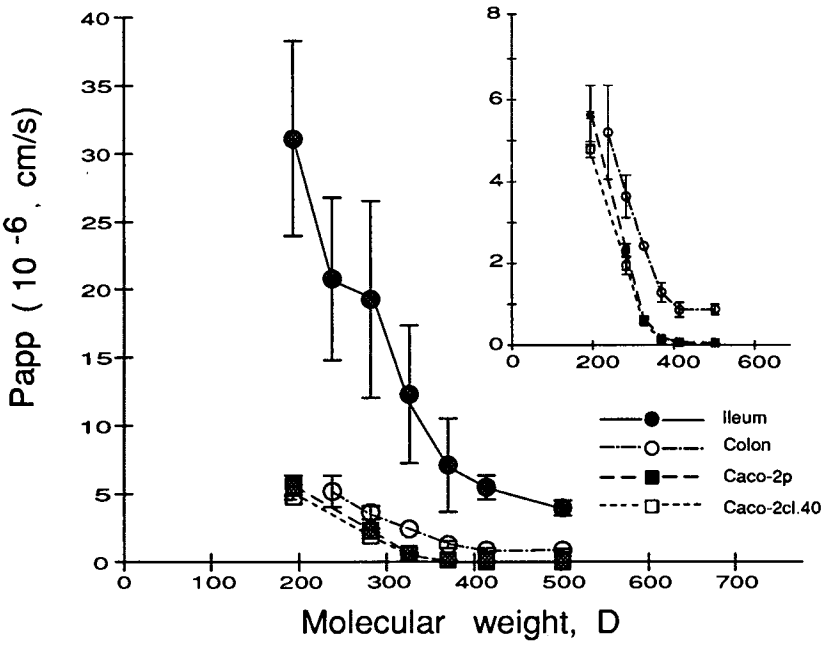

Fig. 1. Apparent permeability coefficients of monodisperse polyethylene glycols of different molecular weights in rat ileum, rat colon, Caco-2p, and Caco-2cl.40. The inset is an enlargement that allows comparison of the apparent permeability coefficients in rat colon and Caco-2 cells. Values represent the mean ± SD (n = 3–6 for each point).

were in good agreement. This indicates that the selective barrier function of the tight junctions in the different *in vitro* models was comparable to that of the intact human intestine, a statement supported by recent results showing a very good correlation between drug permeability in the Caco-2 model and intestinal drug absorption in humans (12).

The permeabilities of different hydrophilic marker molecules have often been assumed to be inversely correlated to

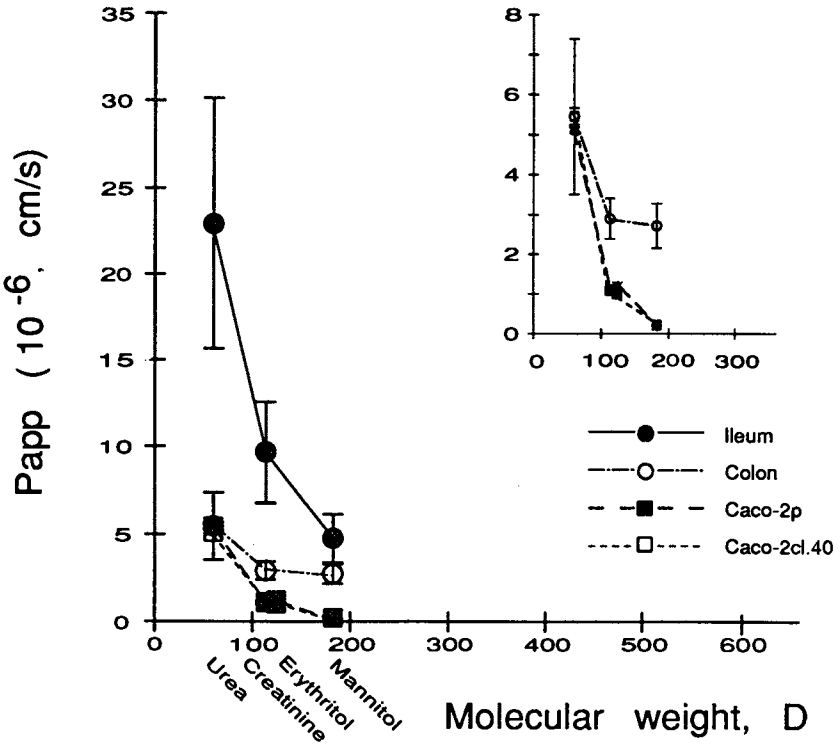

Fig. 2. Apparent permeability coefficients of urea, creatinine, erythritol, and mannitol in rat ileum, rat colon, Caco-2p, and Caco-2cl.40. The inset is an enlargement that allows comparison of the apparent permeability coefficients in rat colon and Caco-2 cells. Values represent the mean ± SD (n = 3–6 for each point).

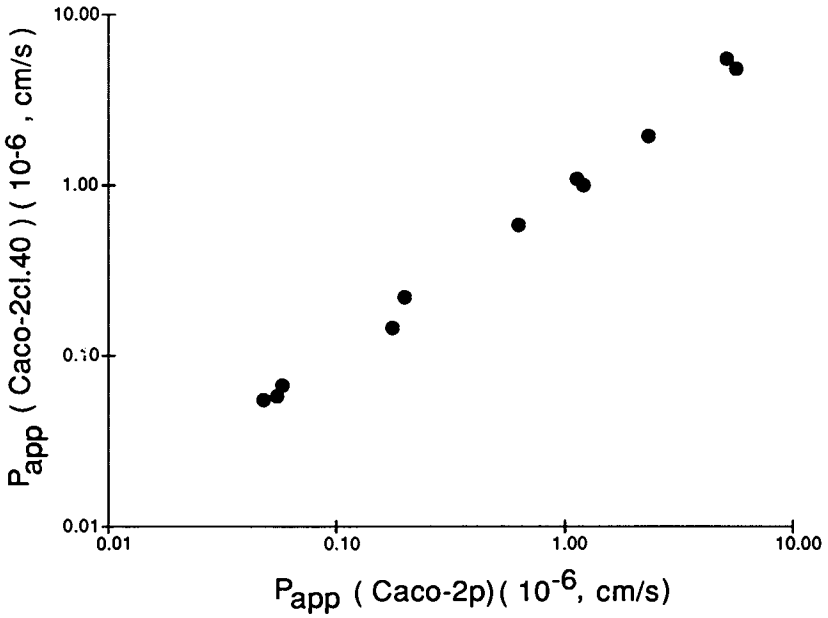

Fig. 3. Correlation of apparent permeability coefficients in Caco-2p and Caco-2cl.40.

their molecular weights. However, our results show that the permeabilities of the PEGs were higher than the permeabilities of the other molecules. For instance, in the different models the permeability of the PEG with a molecular weight of 194 g/mol was 6- to 28-fold higher than the permeability of mannitol (M_w, 182 g/mol). Thus, two permeability profiles appeared when the permeability coefficients were represented as a function of molecular weight. The use of the hydrodynamic radius instead of molecular weight in the profile graphs did not compensate for this difference. These findings indicate that the permeabilities of the different marker molecules are not simply inversely proportional to their molecular weight or molecular radii. A likely explanation to the appearance of two permeability profiles (rather than one) is given by the differences in molecular structure between the PEGs and the other molecules. The PEGs are small and flexible polymers and therefore possible changes in their molecular dimensions and modes of Brownian motion should be considered, e.g., reptation motion (25), through the narrow tight junctions. However, whether these considerations should be based on cross-sectional diameter (5) or on the radius of gyration (26) is an open question. If cross-sectional diameter were used, the treatment would assume that all PEGs are completely stiff, i.e., straight chains. On the contrary, other hydrophilic marker molecules, including those used in this study (urea, erythritol, creatinine, and mannitol), as well as those used by others (e.g., ^{51}Cr-EDTA, lactulose, raffinose, rhamnose), can be considered to span

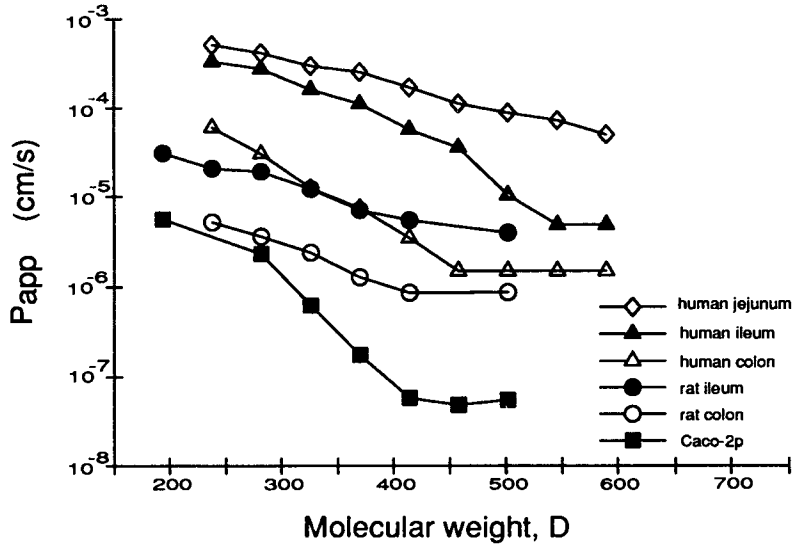

Fig. 4. Calculated apparent permeability coefficients for polyethylene glycols of different molecular weight in human jejunum, ileum, and colon compared with values obtained from the measurements in this study. Calculations of permeability coefficients for the human data were made from the results of Chadwick *et al.* (4).

negligible changed molecular volumes during their passage through a restricted space such as the tight junction. Therefore, suggestions about simple relationships between molecular size parameters and permeability should be treated with caution.

For all permeability profiles, the molecular weight dependence could be divided into two phases. In the case of the PEGs, a more pronounced permeability dependence on molecular weight could be observed for molecular weights of approximately <400 g/mol. A molecular weight cutoff of 400–500 g/mol is in agreement with data reported in several other species including man and gives further support to the statement that the *in vitro* models used in this study have a selective barrier function similar to that found *in vivo* (1,4,17). However, the tight junction can be regulated by hormones and nutrients (e.g., Refs. 6 and 23). Therefore, under some physiological conditions the permeability through the tight junctions can be altered. It is not yet known how such alterations affect the permeability profiles in the *in vitro* models as compared to *in vivo*.

An alternative method for the characterization of the selective barrier function of intestinal epithelium is to measure the transepithelial electrical resistance (TEER) (2). The resistance values for the rat tissues and cell monolayers were in agreement with the literature and increased in the order rat ileum < rat colon < Caco-2p < Caco-2cl.40 (2,10,15). TEER correlates with the permeability of marker molecules such as mannitol (24); however, there was not a strong linear correlation between these measurements. Possibly, the smaller marker molecules are similar in size to the ions + hydration shell that determine the TEER (mainly sodium and chloride ions).

The major difference, however, between TEER and marker permeability measurements is that the resistance reflects the total tissue resistance to ion flow (i.e., both passive paracellular and transcellular ion flow). In more leaky epithelia, such as small intestinal epithelium, the passive paracellular ion flow dominates and the transcellular ion flow can be neglected. However, in tighter epithelia such as the colon, the passive paracellular ion flow is reduced and the transcellular ion flow is significant (2). Therefore, in colonic tissues, the TEER is influenced significantly by the transcellular ion flux. A difference in transcellular ion flux would explain the discrepancy between the measured TEER and the permeabilities of the marker molecules in the two cell lines. Thus, e.g., Caco-2p. had a significantly lower resistance than Caco-2cl.40, but the permeability profiles for the marker molecules were identical in the two cell lines. These results also indicate that the permeability to marker molecules gives a more correct description than TEER of the selective barrier function in tighter epithelia, such as Caco-2 cell monolayers.

In conclusion, the results of this study indicate that both excised ileal and colonic segments from the rat and monolayers of Caco-2 cells have permeability profiles that are comparable to those found in the normal human intestine *in vivo*. These results emphasize the usefulness of these models in drug absorption studies.

ACKNOWLEDGMENTS

This work was supported by grants from Astra Hässle AB, the Swedish Medical Research Council (B91-04X-09478-01A), The Swedish Fund for Scientific Research Without Animal Experiments, and Centrala Försöksdjursnämnden. The skillful technical assistance of M. Lakpour in some of the experiments is also gratefully acknowledged.

REFERENCES

1. D. Hollander. The intestinal permeability barrier. A hypothesis as to its regulation and involvement in Crohn's disease. *Scand. J. Gastroenterol.* 27:721–726 (1992).
2. D. W. Powell. Intestinal water and electrolyte transport. In L. R. Johnson (ed), *Physiology of the Gastrointestinal Tract,* Raven Press, New York, 1987, pp. 1267–1305.
3. G. Davis, C. Santa Ana, S. Morawski, and J. Fordtran. Permeability characteristics of human jejunum, ileum, proximal colon and distal colon: Results of potential difference measurements and unidirectional fluxes. *Gastroenterology* 83:844–850 (1982).
4. V. S. Chadwick, S. F. Phillips, and A. F. Hofmann. Measurements of intestinal permeability using low molecular weight polyethylene glycols (PEG 400). II. Application to normal and abnormal permeability states in man and animals. *Gastroenterology* 73:247–251 (1977).
5. D. Hollander, D. Ricketts, and C. A. R. Boyd. Importance of "probe" molecular geometry in determining intestinal permeability. *Can. J. Gastroenterol.* 2 (suppl. A):35A–38A (1988).
6. J. R. Pappenheimer. Physiological regulation of transepithelial impedance in the intestinal mucosa of rats and hamsters. *J. Membr. Biol.* 100:137–148 (1987).
7. S. G. Schultz. A cellular model for active sodium absorption by mammalian colon. *Annu. Rev. Physiol.* 46:435–451 (1984).
8. A. L. Ungell, A. Andreasson, K. Lundin, and L. Utter. Effects of enzymatic inhibition and increased paracellular shunting on the transport of vasopressin analogs in the rat. *J. Pharm. Sci.* 81:1–6 (1992).
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. G. Wilson, I. F. Hassan, C. J. Dix, I. Williamson, R. Shah, and M. Mackay. Transport and permeability properties of human Caco-2 cells: An in vitro model of the intestinal epithelial cell barrier. *J. Control. Release* 11:25–40 (1990).
11. 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).
12. 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. Comm.* 175(3):880–885 (1991).
13. J. Fogh, J. M. Fogh, and T. J. Orfeo. One hundred and twenty seven cultured human tumor cell lines producing tumors in nude mice. *J. Natl. Cancer Inst.* 59:221–226 (1977).
14. C. H. von Bonsdorff, S. D. Fuller, and K. Simons. Apical and basolateral endocytosis in Madin-Darby canine kidney (MDCK) cells grown on nitrocellulose filters. *EMBO J.* 4:2781–2792 (1985).
15. S. Woodcock, I. Williamson, I. Hassan, and M. Mackay. Isolation and characterisation of clones from the Caco-2 cell line displaying increased taurocholic acid transport. *J. Cell. Sci.* 98:323–332 (1991).
16. J. Karlsson and P. Artursson. A method for the determination of cellular permeability coefficients and aqueous boundary layer thickness in monolayers of intestinal epithelial (Caco-2) cells grown in permeable filter chambers. *Int. J. Pharm.* 71:55–64 (1991).
17. C. Tagesson and R. Sjödahl. Passage of molecules through the wall of the gastrointestinal tract. Urinary recovery of different-sized polyethylene glycols after intravenous and intestinal deposition. *Scand. J. Gastroenterol.* 19:315–320 (1984).
18. I. Komiya, J. Y. Park, A. Kamani, N. F. H. Ho, and W. I. Higuchi. Quantitative mechanistic studies in simultaneous fluid flow

and intestinal absorption using steroids as model solutes. *Int. J. Pharm.* 4:249–262 (1980).
19. J. R. Pappenheimer. Paracellular intestinal absorption of glucose, creatinine, and mannitol in normal animals: Relation to body size. *Am. J. Physiol.* 259:G290–G299 (1990).
20. M. A. Marcial, S. L. Carlson, and J. L. Madara. Partitioning of paracellular conductance along the ileal crypt-villus axis: A hypothesis based on structural analysis with detailed consideration of tight junction structure-function relationships. *J. Membr. Biol.* 80:59–79 (1984).
21. J. L. Madara and J. S. Trier. Structure and permeability of goblet cell tight junctions in rat small intestine. *J. Membr. Biol.* 66:145–157 (1982).
22. L. Johansson, U. Skantze, and J. E. Löfroth. Diffusion and interaction in gels and solutions. II. Experimental results on the obstruction effect. *Macromolecules* 2:6019–6023 (1991).
23. J. A. McRoberts, R. Aranda, N. Riley, and H. Kang. Insulin regulates the paracellular permeability of cultured intestinal epithelial cell monolayers. *J. Clin. Invest.* 85:1127–1134 (1990).
24. J. L. Madara and K. Dharmsathaporn. Occluding junction structure-function relationships in a cultured epithelial monolayer. *J. Cell Biol.* 101:2124–2133 (1985).
25. H. Fujita. Polymer solutions. In *Studies in Polymer Science, Vol. 9,* Elsevier, Amsterdam, 1990.
26. L. Johansson, C. Elvingson, and J. E. Löfroth. Diffusion and interaction in gels and solutions. III. Theoretical results on the obstruction effect. *Macromolecules* 24:6024–6029 (1991).