Comparison Between Permeability Coefficients in Rat and Human Jejunum

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Received March 26, 1996; accepted May 28, 1996

Purpose. Our main aim is to determine the effective intestinal permeability (P_{eff}) in the rat jejunum *in situ* for 10 compounds with different absorption mechanisms and a broad range of physico chemical properties, and then compare them with corresponding historical human *in vivo* P_{eff} values.

Methods. The rat P_{eff} coefficients are determined using an *in situ* perfusion model in anaesthetized animals. The perfusion flow rate used is 0.2 ml/min, which is 10 times lower than that used in humans. The viability of the method is assessed by testing the physiological function of the rat intestine during perfusions.

Results. The P_{eff} for passively absorbed compounds is on average 3.6 times higher in humans compared to rats ($P_{eff,man} = 3.6 \cdot P_{eff,rat} + 0.03 \cdot 10^{-4}$; R^2 = 1.00). Solutes with carrier-mediated absorption deviate from this relationship, which indicates that an absolute scaling of active processes from animal to man is difficult, and therefore needs further investigation. The fraction absorbed of drugs after oral administration in humans (fa) can be estimated from $1 - e^{-(2 \cdot P_{eff,man} \cdot t_{res} / r - 2.8)}$.

Conclusions. Rat and human jejunum P_{eff}-estimates of passively absorbed solutes correlate highly, and both can be used with precision to predict *in vivo* oral absorption in man. The carrier-mediated transport requires scaling between the models, since the transport maximum and/or substrate specificity might differ. Finally, we emphasize the absolute necessity of including marker compounds for continuous monitoring of intestinal viability.

KEY WORDS: bioavailability; *in situ-in vivo* correlation; intestinal perfusion; intestinal permeability; oral absorption.

INTRODUCTION

Investigation of the delivery of systemically acting drugs across the intestinal membrane is fundamental to the development of strategies for improving of the amount of drug absorbed from products intended for oral use, and requires increased understanding of the basic mechanisms involved in the membrane translocation of drugs across the intestinal epithelium. Thus, different models (e.g., *in situ* animal and cell culture models) must be used and thoroughly compared with corresponding human variable(s). The transmucosal transport of most drugs *in vivo* is thought to occur by passive diffusion, where the cell membrane is rate limiting, and not the unstirred water layer adjacent to the intestinal wall (1). The passive transmucosal diffusion is best described by the effective intestinal permeability (P_{eff}, cm/s), which is determined by the membrane/lumen partitioning (K) and the membrane diffusion coefficient (D_m) ($P_{eff} = K \cdot D_m/\lambda$: "Overton's rule") (2). λ represents the transport distance for a molecule across the apical intestinal cell membrane, which in the human jejunum is approximately 10 nm (3). The general view is that lipophilic compounds are absorbed by the transcellular pathway, whereas hydrophilic and charged molecules which have lower P_{eff}, due to low K and/or D_m-values, are transported through the water filled space between the epithelial cells, the so called paracellular route (4). However, the quantitative importance of drug transport by the paracellular route *in vivo* in man and rat has been questioned recently (5–10). Instead, the transcellular route was suggested to be the dominating absorption route in quantitative aspects, regardless of the physicochemical properties of the drug. Furthermore, recent data indicate that high levels of nutrients, such as D-hexoses and/or L-amino acids, in the intestinal lumen do not affect the passive para- and/or transcellular drug absorption in man and rat (5–7,9). The high nutrient levels did neither increase intestinal water absorption *in vivo* in man (5–7).

One of the major aims of the current study is to determine P_{eff} in the rat jejunum for 10 compounds with different absorption mechanisms and a broad range of physico chemical properties, and then compare them with corresponding human P_{eff} values. The human permeability data have been published elsewhere and originate from our previous research in which the regional perfusion approach was applied in the human proximal jejunum (5–7, 11–14). These human P_{eff}-values correlate well to the extent of absorption in man (7, 22). The animal permeability coefficients are determined using an *in situ* perfusion model in anaesthetized rats. The perfusion flow rate used in these animal experiments (0.2 ml/min) is 10 times lower than in humans (2–3 ml/min). Earlier studies have shown that the extent of absorption in humans can be predicted from an intestinal permeability value obtained by *in situ* perfusion in rats (15). However, unlike that particular study, we directly compare the quantitative difference between the two models. Furthermore, we compare the *in situ* model with an *in vivo* model in man, since it is essential in order to obtain the physiological relevance of the *in situ* data (10). Another major purpose is to estimate a correction factor, which is essential for obtaining a robust estimate of fraction absorbed (fa) *in vivo* in man. We also predict how changes of the interplay between P_{eff} and small intestinal transit time (t_{res}) will affect the fa in humans. In addition, our aim is to the validate the *in situ* model with regard to physiological functional assessment by using three different marker compounds, PEG 4000, D-glucose and antipyrine.

MATERIALS AND METHODS

Single-pass Perfusion of the Rat Jejunum

Male Sprague-Dawley rats weighing approximately 200–270 g were fasted for 12–20 h (water ad libitum) prior to each experiment. Anaesthesia was induced with an i.p. injection of Inactin®-Byk (thiobutabarbital sodium: 120 mg/kg). To maintain normal body temperature rats were placed on a heated slide warmer and under a heating lamp. The temperature was measured in the segment and was found to be between 36 and 37°C. Breathing was facilitated by the introduction of a plastic tube into the trachea. The abdomen was opened with a midline

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incision and a jejunal segment of approximately 10 cm was measured, isolated and cannulated with plastic tubing (4 mm o.d., inlet tube 40 cm, outlet tube 25 cm). Care was taken to avoid disturbance of the circulatory system, and the exposed segment was kept moist with body tempered saline. Initially, the intestinal segment was rinsed with isotonic saline (37°C) until the outlet solution was clear. The preparation time took approximately 30 minutes. A bolus dose of 3 ml of solution containing the substance(s) of interest was given and thereafter followed by a constant perfusion at a flow rate (Q_{in}) of 0.2 ml/min (Harvard Apparatus 22). Each perfusion experiment lasted for 100 min, and perfusate was quantitatively collected in the following intervals; 0–20, 20–40, 40–55, 55–70, 70–85 and 85–100 min. At the end of the perfusion the intestinal segment was rinsed with approximately 15 ml saline for 2–5 minutes in order to collect the remaining amounts of substances from the perfusion system. All perfusion syringes and perfusate samples were weighed and the samples were frozen immediately and stored at −20°C until analysis. Approval for these animal studies was given by the Animal Ethics Committee of the Medical Faculty, Uppsala University (application number C303/92).

Single-pass Perfusion of the Human Jejunum

Single-pass perfusions (Loc-I-Gut®) were carried out in the proximal jejunum in healthy fasting subjects, and the perfusion flow rate was 2.0–3.0 ml/min (11).

Composition of the Perfusion Solutions

Rat jejunal P_{eff}-values of 10 different compounds were determined and compared to those recently obtained in humans (5–7, 11–14). Information about the physico chemical characteristics (MW, pKa, log D), inlet perfusate concentrations (C_{in}) and number of experiments (n) of each compound is shown in Table I. The composition of the perfusion solution was as follows: NaCl (48 mM), KCl (5.4 mM), Na_2HPO_4 (28 mM), NaH_2PO_4 (43 mM), mannitol (35 mM), polyethylene glycol (PEG 4000: MW 4000: 1 g/l) and D-glucose (10 mM). ^{14}C-labelled polyethylene glycol [^{14}C]PEG 4000 (2.5 μCi/l) (Amersham Labs., Buckinghamshire, England) was added to the solution as a non-absorbable marker. Antipyrine (1.05 mM) was used as a reference probe for passive absorption. The pH and osmolality of the inlet perfusion solution was kept at 6.5 and 290 mOsm/l, respectively, with the exception of the experiments in which levodopa was studied (pH 7.4). Atenolol, furosemide, ketoprofen, levodopa, metoprolol, naproxen and terbutaline were studied in separate experiments, and always in combination with antipyrine, D-glucose and PEG 4000. L-dopa was administered together with the decarboxylase inhibitor, benserazide.

Stability and Adsorption Tests

The stability of antipyrine, atenolol, D-glucose, furosemide, ketoprofen, L-dopa, metoprolol, naproxen and terbutaline has earlier been assessed by incubation at 37°C for 150 min (5, 7, 11–14). There was no sign of degradation of these solutes during this period of time, and none was adsorbed to the catheters.

Functional Viability Tests

The functional viability of the intestinal tissue is always crucial when *in situ* models for intestinal membrane transport

Table I. Physico Chemical Properties (molecular weight, MW; pKa, partition between Octanol/Water at pH 7.4; log D), Inlet Concentrations (C_{in}), and Number of Experiments (n) of 10 Different Compounds Studied by Jejunal Perfusion in the Rat and Man. The pH and Osmolality of the Solutions Entering the Test Segments were 6.5 and 290 mOsm/L, Respectively, Except for the Experiments with Levodopa Where a pH of 7.4 Was Used. Antipyrine and PEG 4000 Were Included in all Experiments as Reference and Volume Marker Probes, Respectively

	Physico chemical parameters		*Experimental information*		
	MW [g/mole]	*pKa*	*log D* [oct/water, pH 7.4*]	*Cin* [mM]	*n* [rat/man]
Passive absorption					
Antipyrine	188	1.5[a] (base)	0.4[b]	1.05	58/75[h–k]
Atenolol	266	9.6[b] (base)	−1.8[a]	0.83	12/8[a]
Furosemide	331	3.8[c] (acid)	−0.8[d]	0.20	6/8[l]
Ketoprofen	254	4.6[c] (acid)	0.3[e]	0.67	6/8[l]
Metoprolol	267	9.7[b] (base)	0.0[f]	0.58	11/8[m]
Naproxen	230	4.4[b] (acid)	0.1[e]	1.8	7/8[l]
PEG 4000	4000	— (—)	n.i.	0.25	58/75[h–k]
Terbutaline	225	8.8, 10.1, 11.2[c] (base)	−1.4[g]	0.010	8/15[k]
Carrier-mediated absorption					
D-glucose	180	— (—)	−3.0[d]	10	12/53[h,i,k]
L-dopa	197	2.3, 8.7, 9.7, 13.4[b] (acid)	n.i.	2.5	8/22[i,j]

Note: n.i.; no information available, *log D-estimates of ketoprofen and naproxen are recalculated values.

[a] Taken from Ref. 5.
[b] Taken from Ref. 28.
[c] Taken from Ref. 29.
[d] Taken from Ref. 30.
[e] Taken from Ref. 31.
[f] Taken from Ref. 32.
[g] Taken from Ref. 33.
[h] Taken from Ref. 11.
[i] Taken from Ref. 12.
[j] Taken from Ref. 6.
[k] Taken from Ref. 7.
[l] Taken from Ref. 14.
[m] Taken from Ref. 13.

are used. In this study we performed functional assessment of the intestinal barrier by using different marker compounds such as [^{14}C]PEG 4000, D-glucose and antipyrine. PEG 4000 labelled with ^{14}C is an established non-absorbable compound and was used as a marker for an intact jejunal barrier. Further validation of the model was obtained by investigating the carrier-mediated cotransport of Na^+/D-glucose. This Na^+/D-glucose cotransporter is a membrane protein (SGLT1) that is crucial for the membrane transport of these two compounds. Antipyrine was included as a marker for passive transcellular absorption, and has been widely used in our human regional perfusion experiments. The P_{eff} of antipyrine is also used as an indicator of extensive changes of mesenteric blood flow (16).

Analytical Methods

All chemicals used were of analytical grade. Antipyrine, atenolol, L-dopa, and terbutaline were assayed by previously validated and used h.p.l.c. methods (5, 7, 11, 12). The following drugs were analysed at the Medical Product Agency, Uppsala, Sweden: furosemide, ketoprofen, metoprolol and naproxen. The limits of quantification of furosemide, ketoprofen, metoprolol, and naproxen were 0.9, 5.0, 4.5 and 22.5 μg/ml, respectively. The corresponding variability of LOQ, expressed by coefficient of variation, for each drug was 4.9, 5.1, 9.5 and 14.7%, respectively. Levels of D-glucose were determined by using an automatic multi-analysing instrument (Hitachi 717, Boehringer Mannheim) and levels of [^{14}C]PEG 4000 by liquid scintillation counting for 10 min (Beckman instrument, model 244) after addition of 8 ml Beckman Ready Safe®. The osmolality of the perfusion solutions was measured by the vapor pressure method (Vescor osmometer 5500).

Data Analysis

Calculations were made from the steady-state concentrations of the outlet perfusate, which was considered to have been achieved when the concentration level of [^{14}C]PEG 4000 was stable (between 40–100 min after the start of perfusion). The net water flux (NWF) per cm of the segment was calculated using equation 1:

$$NWF = \frac{(1 - [PEG]_{out} / [PEG]_{in}) \cdot Q_{in}}{L} \quad (1)$$

where $[PEG]_{in}$ and $[PEG]_{out}$ were the inlet and outlet concentrations of [^{14}C]PEG 4000, respectively. Q_{in} was the flow rate of the perfusion solution entering the intestinal segment, and L was the length of the segment (10 cm). At a Q_{in} of 2–3 ml/min in humans we have clearly demonstrated that P_{eff} is membrane controlled, and not affected by UWL (1). In the rat model we scaled the Q_{in} to 0.2 ml/min, since the radius of the rat intestine is about 10 times less than in humans. According to a residence time distribution analysis are the hydrodynamics and concentration-time profile within the human intestinal segment best described by the well-mixed model (17, 18). Considering the rat model, we used the same approach as other groups (the parallel-tube model) (19, 20). The effective intestinal permeability (P_{eff}) was calculated (11, 18, 20):

$$P_{eff,man} = \frac{Q_{in} \cdot (C_{in} - C_{out}) / C_{out}}{2\pi rL} \quad (2)$$

$$P_{eff,rat} = \frac{-Q_{in} \cdot \ln (C_{out} / C_{in})}{2\pi rL} \quad (3)$$

where C_{in} and C_{out} were the inlet and outlet (fluid transport-corrected) concentrations of each compound, respectively. $2\pi rL$ is the mass transfer surface area within the intestinal segment which is assumed to be the area of the cylinder with the length (L) of 10 cm and a radius (r) of 1.75 cm in man and 0.18 cm in the rat, respectively (Knutson, L. personal communication, 20). The recovery of [^{14}C]PEG 4000 (PEG_{rec}) was:

$$PEG_{rec} = \Sigma PEG_{out} / \Sigma PEG_{in} \quad (4)$$

where ΣPEG_{in} and ΣPEG_{out} were the accumulated amounts of [^{14}C]PEG 4000 entering and leaving the intestinal segment during equilibrium, respectively. The complete radial mixing (parallel tube) model (CRM) was used to predict the fraction absorbed *in vivo* in man ($fa_{,pred}$) (21).

$$fa_{,pred} = 1 - e^{-(2 \cdot P_{eff} \cdot t_{res} / r \cdot f)} \quad (5)$$

where t_{res} and r are the average small intestinal transit time and radius in humans, and are assumed to be 3 hrs and 1.75 cm, respectively (Knutson, L. personal communication, 22). The relationship between our human P_{eff} and fa (from *in vivo* pharmacokinetic studies), expressed as the correction factor *f*, was determined by non-linear regression (MINIM 3.0 by R.D Purves) (23). The *f*-value was then used for simulation and prediction of fa *in vivo* in man (equation 5, Figure 3). Throughout this paper variability is expressed as standard deviation (SD).

RESULTS AND DISCUSSION

The steady-state values of the effective permeability coefficients of both D-glucose and antipyrine throughout the animal perfusion experiment were stable and demonstrate low variability (C.V. ≈10–15%), indicating that the jejunal epithelial cells in the rat model possess normal mucosal transport properties (both passive and active) and metabolic functions. Furthermore, the stable transmucosal transport of antipyrine indicates that no dramatic changes in mesenteric blood flow occurred over time in these anaesthetized rats (16). The total recovery of PEG 4000 was 99 ± 4.8% indicating that the intestinal mucosa in the rats was intact. These validation criteria in the animal model agree with our human regional perfusion approach for the same marker compounds (11, 12). Furthermore, our rat P_{eff} data are consistent with those obtained by others during both anaesthetic and nonanaesthetic conditions (24, 25) (Table II). Based on these observations for the three marker molecules, we consider our *in situ* perfusion model validated for investigations of drug absorption mechanisms.

The importance of including these viability markers was clearly demonstrated in previous rat experiments performed in our laboratory. We found an extensive uptake of the nonabsorbable volume marker [^{14}C]PEG 4000 (30% absorption) and atenolol (P_{eff} = 0 to $1.3 \cdot 10^{-4}$ cm/s and fa = 0 to 35%) (Table II). We believe that this finding represents unphysiological leakage across the jejunal mucosa. Both compounds have low partitioning into lipid membranes, and their absorption is therefore probably highly affected by a disruption of membrane

Table II. The Effective Permeability Values of Different Viability Marker Compounds in the *in Situ* Rat Perfusion Model. The Table Includes Previously Reported Data for Comparison and Validation

	Poor functional viability		*Normal functional viability*		
PEG recovery [%]	75 ± 7.7	(P<0.0001)	99 ± 4.8		
Peff [$\cdot 10^{-4}$ cm/s]					
Antipyrine	1.4 ± 0.44		1.6 ± 0.40		
D-glucose	1.3 ± 0.43		1.3 ± 0.40		
Atenolol	0.90 ± 0.57	(P<0.01)	0.055 ± 0.074		
	Non-anaesthesia[a]	*Pentobarbital*[a]	*Urethane*[a]	*Urethane*[b]	*Thiobutabarbital (our data)*
Peff [$\cdot 10^{-4}$ cm/s]					
Antipyrine	—	—	—	1.3 ± 0.1	1.6 ± 0.40
D-glucose (1 mM)	2.7 ± 0.51	1.8 ± 0.26	0.99 ± 0.27	—	—
D-glucose (10 mM)	—	—	—	—	1.3 ± 0.40
D-glucose (100 mM)	0.95 ± 0.17	0.72 ± 0.22	0.42 ± 0.12	—	—
Urea	0.56 ± 0.13	0.46 ± 0.12	0.35 ± 0.07	0.6 ± 0.03	0.87 ± 0.14[c]
Water (^3H_2O)	2.3 ± 0.20	2.2 ± 0.51	1.1 ± 0.20	2.2 ± 0.2	—
Water (D_2O)	—	—	—	—	2.0 ± 0.14[c]

[a] Taken from Ref. 24 (recalculated mean estimates).
[b] Taken from Ref. 25 (one-compartment model with perfect luminal mixing).
[c] Taken from Ref. 27.

integrity. We also found that antipyrine has an unchanged P_{eff} in these rats, which is in agreement with its relatively small size (MW 188), sufficiently high log D and P_{eff} values (Table I). Therefore, its absorption is assumed to be less affected by a loss of integrity of the jejunal barrier (Table II). A change in the intestinal barrier function is probably one of the main reasons for several of the contradictory results that have been obtained by *in vitro* and *in situ* models concerning intestinal permeability of drugs (8, 10, 26).

Intestinal absorption is a complex process where not all underlying mechanisms are fully understood. Several factors, such as for example, intestinal disease, surgery and the choice of anaesthetic might influence the P_{eff} (9, 24). For instance, it was recently reported that surgery and anaesthetics might cause anoxia and decreases in intestinal blood flow, motility and energy supply, with consequent decreases of both passive and active transport (9, 24). Anaesthetics might also have direct effect(s) on cell membranes.

According to the data shown in Table II, barbiturates (including thiobutabarbital which was used in the present study) seem to have the least effect upon intestinal drug absorption. Our permeability data are similar to those obtained during non-anaesthesia conditions (24), which support a validation of the *in situ* model to *in vivo* conditions. This is further supported by the fact that high levels of nutrients within the intestinal lumen and/or an induced intestinal water absorption had no effect on the P_{eff} of atenolol and PEG 4000 in rats, and terbutaline, atenolol, enalaprilat, antipyrine, D-glucose and PEG 4000 *in vivo* in man (5–7,9,27).

The absorption parameters and the rank order of the P_{eff}-estimates are presented in Table III. For passively absorbed compounds the rank order was the same in both species. There was a high correlation ($P < 0.0001$) between the two models ($P_{eff,man} = 3.6 \cdot P_{eff,rat} + 0.03 \cdot 10^{-4}$; R^2 = 1.00), with an intercept not significantly different from zero (Fig. 1). The human P_{eff}-estimates for all drugs absorbed by passive diffusion were on average 3.6 times higher than in the rat irrespective of the permeability classification of the drug (Fig. 1). Plausible reasons for the lower value in the rat model are differences in effective absorptive area within the perfused segment, and/or species differences affecting partitioning into the membrane (K), diffusion coefficient (D_m) and/or diffusion distance (λ). Carrier-mediated transported compounds (such as L-dopa and D-glucose) deviate from this linear relationship (Table III and Figure 1), which clearly demonstrates that each actively transported drug has to be carefully investigated in order to find the accurate mechanism(s) and a scaling factor.

Both human and rat P_{eff}-values predict the quantitative amount of drug absorbed *in vivo* in man very well, when given as a solution and/or IR-dosage form, i.e., when the drug is absorbed in the proximal part of the small intestine (Table III and Figure 2). The best fit for the data (human P_{eff} vs fa) was observed when f was 2.8 ± 0.19. Consequently, fa can be predicted from $1 - e^{-(2 \cdot P_{eff,man} \cdot t_{res}/r \cdot 2.8)}$ in humans and $1 - e^{-[2 \cdot (P_{eff,rat} \cdot 3.6 + 0.03 \cdot 10^{-4}) \cdot t_{res}/r \cdot 2.8]}$ in the rat, respectively. The f-value is thought of as a proportionality constant for effective permeability values between *in vivo* perfusion and oral bioavailability studies. The plausible reasons for the deviation of our estimate (f = 2.8) from 1 are: (a) variable intestinal transit time; (b) variation in the exposed absorbing intestinal area; (c) regional differences in absorption; (d) regional variation in the hydrodynamics which might influence fa *in vivo;* and (e) the steep relationship between P_{eff} and fa creates uncertainity in fitting the model.

Research in oral drug delivery requires estimating how changes in P_{eff} and t_{res} in the experimental model will affect the fraction absorbed *in vivo* quantitatively. In Fig. 3 is demonstrated the effect a change in human P_{eff} and/or t_{res} might have on the fa *in vivo* in man. Based on this relationship, with a t_{res} of 3 hours, three different classes of *in vivo* absorption may be defined in man: (a) poor, 0–30%; (b) intermediate, 30–90%; (c) and complete, 90–100% (Fig. 3). According to this classification (t_{res} = 3 hours), compounds with an average P_{eff} throughout the small intestine in humans of approximately $<0.1 \cdot 10^{-4}$ cm/s are poorly

Table III. Absorption Parameters of 10 Different Compounds Studied by Jejunal Perfusion in the Rat and Man, Respectively. The P_{eff} Values Are Presented as the Mean ± SD

	Effective permeability, Peff [·10-4 cm/s] (Experimental)			Fraction absorbed in humans, fa [%] (Literature)	(Predicted)	
Compound	Rat	Man	Rank Order* rat/man	observed *in vivo* in man	from human P_{eff}**	from rat P_{eff}**
Passive absorption						
Antipyrine	1.6 ± 0.40	5.6 ± 1.6[a–e]	3/3	100[a]	100	100
Atenolol	0.055 ± 0.074	0.15 ± 0.2[c]	6/7	50[h,i]	40	52
Furosemide	0.13 ± 0.082	0.3 ± 0.3[f]	5/5	60[h,j,k]	64	80
Ketoprofen	2.4 ± 0.55	8.5 ± 3.9[f]	1/1	100[i]	100	100
Metoprolol	0.33 ± 0.20	1.5 ± 0.9[g]	4/4	≥95[h,i,l]	99	98
Naproxen	2.1 ± 0.41	8.0 ± 4.2[f]	2/2	100[h]	100	100
PEG4000	≈0	≈0[a–d]	8/8	0[m]	0	0
Terbutaline	0.045 ± 0.080	0.3 ± 0.3[e]	7/5	60[e,j]	64	45
Carrier-mediated absorption						
D-glucose	1.3 ± 0.40	11 ± 8.2[a,b,e]	—/—	100	100	***
L-dopa	2.0 ± 0.63	3.4 ± 1.7[b,d]	—/—	≈100[b]	100	***

Note: *Compounds utilizing carrier-mediated transport are not ranked. **fa was predicted from $1 - e^{-2 \cdot P_{eff,man} \cdot t_{res}/r \cdot 2.8}$ and $1 - e^{-2 \cdot (3.6 \cdot P_{eff,rat} + 0.03 \cdot 10^{-4}) \cdot t_{res}/r \cdot 2.8}$ in man and rat, respectively. t_{res} was set to 3 hours and r to 1.75 cm. ***fa values for compounds utilizing carrier-mediated transport were not predicted from rat P_{eff} data.

[a] Taken from Ref. 11.
[b] Taken from Ref. 12.
[c] Taken from Ref. 5.
[d] Taken from Ref. 6.
[e] Taken from Ref. 7.
[f] Taken from Ref. 14.
[g] Taken from Ref. 13.
[h] Taken from Ref. 29.
[i] Taken from Ref. 28.
[j] Taken from Ref. 34.
[k] Taken from Ref. 35.
[l] Taken from Ref. 32.
[m] Taken from Ref. 33.

absorbed, whereas those with P_{eff} of about $>0.7 \cdot 10^{-4}$ cm/s are completely absorbed. Corresponding estimates in rats are $<0.03 \cdot 10^{-4}$ and $>0.2 \cdot 10^{-4}$ cm/s, respectively.

In conclusion, rat and human jejunum P_{eff}-estimates of passively absorbed solutes correlate highly, and both can be used with precision to predict *in vivo* oral absorption in man. Furthermore, the rat model is suitable for predicting how passively absorbed drugs should be classified according to the previously presented biopharmaceutical drug classification system (22). The carrier-mediated transport requires scaling between the models, since the transport maximum and/or substrate specificity might differ. Finally, we emphasize the absolute necessity of including marker compounds for a continuous monitoring of the viability throughout the perfusion experiment, in order to avoid unexpectedly high absorption of espe-

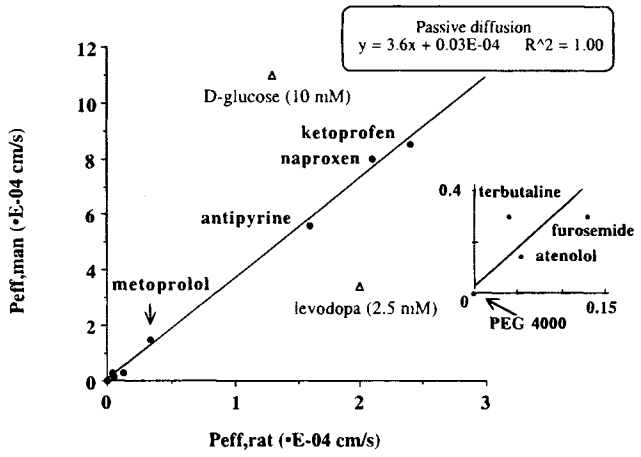

Fig. 1. A comparison between human and rat effective intestinal permeability coefficients (P_{eff}). The equation describes the correlation for passive diffusion. The inset shows the P_{eff}-values in the lower range (● = passive absorption; △ = carrier-mediated absorption).

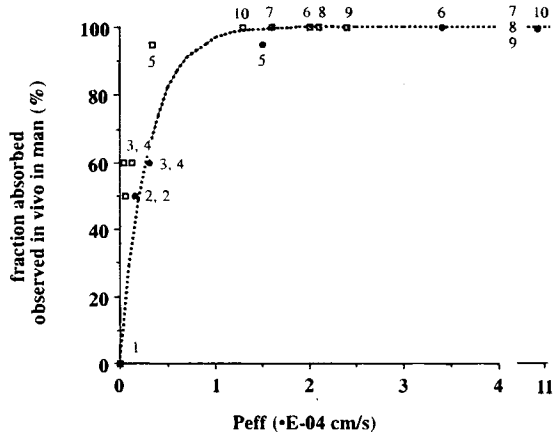

Fig. 2. Human and rat P_{eff}-estimates vs. fraction absorbed (fa) obtained from pharmacokinetic studies *in vivo* in man. (● = human P_{eff}, □ = rat P_{eff}, dotted line = fa predicted from human P_{eff}, $fa = 1 - e^{-(2 \cdot P_{eff,man} \cdot t_{res}/r \cdot 2.8)}$) (1, PEG 4000; 2, atenolol; 3, terbutaline; 4, furosemide; 5, metoprolol; 6, levodopa; 7, antipyrine; 8, naproxen; 9, ketoprofen; 10, D-glucose).

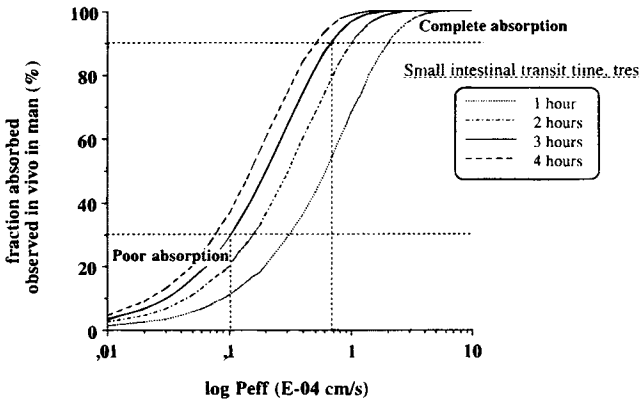

Fig. 3. Theoretical predictions of the relationship between human P_{eff} estimates, small intestinal transit times (t_{res} = 1, 2, 3 and 4 hours) and fraction absorbed *in vivo* in man (fa).

cially low permeability compounds, and to choose a favorable anaesthetic.

ACKNOWLEDGMENTS

We would like to thank Astra Draco AB (Britt-Marie Kennedy and Dr. Lars Borgström) and Astra Arcus AB (Dr. Ulf Bredberg) for financial support. We also thank Dr. Torgny Fornstedt at Medical Products Agency, Uppsala, Sweden, for assistence with some chemical analyses. The skilful technical assistance from Jonas Ödman, Catarina Nilsson, Åsa Nylén, Cecilia Kroon and Charlotta Porath during the rat experiments is also appreciated. This study was also supported in part by the Food & Drug Administration, Rockville, U.S.A. (No. 223 93-303), and by Marcus and Amalia Wallenbergs Minnesfond, Stockholm, Sweden.

REFERENCES

1. U. Fagerholm and H. Lennernäs. Experimental estimation of the effective unstirred water layer thickness in the human jejunum, and its importance in oral drug absorption. *Eur. J. Pharm. Sci.*, **3**:247–53 (1995).
2. A. Walter and J. Gutknecht. Permeability of small nonelectrolytes through lipid bilayer membranes. *J. Membrane Biol.*, **90**:207–17 (1986).
3. J. S. Trier and J. L. Madara. Chapter 35: Functional morphology of the mucosa of the small intestine. In *Physiology of the gastrointestinal tract (2nd edition).* Ed. L. R. Johnson. Raven Press, New York, 1981, pp. 925–61.
4. D. C. Taylor, J. Lynch, and D. E. Leahy. Chapter 11: Models for intestinal permeability to drugs. In *Drug delivery to the gastrointestinal tract,* Ed. J. G. Hardy, S. S. Davis and C. G. Wilson. Chichester, U. K. Ellis Horwood Ltd., 1989, pp. 133–45.
5. H. Lennernäs, Ö Ahrenstedt, and A-L. Ungell. Intestinal drug absorption during induced net water absorption in man: A mechanistic study using antipyrine, atenolol and enalaprilat. *Br. J. Clin. Pharmacol.*, **37**:589–96 (1994).
6. D. Nilsson, U. Fagerholm, and H. Lennernäs. The influence of net water absorption on the permeability of antipyrine and levodopa in the human jejunum. *Pharm. Res.*, **11**:1541–5 (1994).
7. U. Fagerholm, L. Borgström, Ö Ahrenstedt, and H. Lennernäs. The lack of effect of induced net fluid absorption on the *in vivo* permeability of terbutaline in the human jejunum. *J. Drug Targeting,* **3**:191–200 (1995).
8. H. Lennernäs. Does fluid flow across the intestinal mucosa affect quantitative oral drug absorption? Is it time for a reevaluation? *Pharm. Res.*, **12**:1573–82 (1995).
9. M. R. Uhing and R. E. Kimura. Active transport of 3-*O*-methylglucose by the small intestine in chronically catheterized rats. *J. Clin. Invest.*, **95**:2799–805 (1995).
10. J. M. Diamond. News and views: How to be physiological. *Nature,* **376**:117–8 (1995).
11. H. Lennernäs, Ö Ahrenstedt, R. Hällgren, L. Knutson, M. Ryde, and L. Paalzow Regional jejunal perfusion, a new *in vivo* approach to study oral drug absorption in man. *Pharm. Res.*, **9**:1243–51 (1992).
12. H. Lennernäs, D. Nilsson, S-M. Aquilonius, Ö. Ahrenstedt, L. Knutson, and L. Paalzow. The effect of L-leucine on the absorption of levodopa, studied by regional jejunal perfusion in man. *Br. J. Clin. Pharmacol.*, **35**:243–50 (1993).
13. H. Lennernäs, L. Knutson, T. Knutson, L. Lesko, T. Salomonson, and G. L. Amidon. Human effective permeability data for atenolol, metoprolol and carbamazepine to be used in the proposed biopharmaceutical classification for IR-products. *Pharm. Res.*, **12**:295 (1995).
14. H. Lennernäs, L. Knutson, T. Knutson, L. Lesko, T. Salomonson, and G. L. Amidon. Human effective permeability data for furosemide, hydrochlortiazide, ketoprofen and naproxen to be used in the proposed biopharmaceutical classification for IR-products. *Pharm. Res.*, **12**:396 (1995).
15. G. L. Amidon, P. J. Sinko, and D. Fleisher. Estimating human oral fraction dose absorbed: A correlation using rat intestinal membrane permeability for passive and carrier-mediated compounds. *Pharm. Res.*, **5**:651–4 (1988).
16. R. Schultz and D. Winne. Relationship between antipyrine absorption and blood flow rate in rat jejunum, ileum and colon. *Naunyn-Schm. Arch Pharmacol,* **335**:97–102 (1987).
17. I-D. Lee, U. Fagerholm, H. Lennernäs, and G. Amidon. A study of hydrodynamic characteristics in human intestine applying residence time distribution analysis. Urtti Arto Ed. In Symposium on methods to overcome biological barriers in drug delivery. August 26–28, Kuopio University Publications *A Pharm. Sci.*, **10**:p73 (1993).
18. G. L. Amidon, J. Kou, R. L. Elliot, and E. N. Lightfood. Analysis of models for determining intestinal wall permeabilities. *J. Pharm. Sci.*, **69**:1369–73 (1980).
19. M. D. Levitt, J. M. Kneip, and D. G. Levitt. Use of laminar flow and unstirred layer models to predict intestinal absorption in the rat. *J. Clin. Invest.* **81**:1365–9 (1988).
20. 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. Pharmaceutics,* **4**,249–62 (1980).
21. P. J. Sinko, G. D. Leesman, and G. L. Amidon. Predicting fraction absorbed in humans using a macroscopic mass balance approach. *Pharm. Res.*, **8**:979–88 (1991).
22. G. L. Amidon, H. Lennernäs, V. Shah, and J. Crison. A theoretical basis for a biopharmaceutical drug classification: The correlation of *in vitro* drug product dissolution and *in vivo* bioavailability. *Pharm. Res.*, **12**:413–20 (1995).
23. R. D. Purves. MINIM 3.0. A Macintosh application for non-linear parameter estimation, 1994.
24. H. Yuasa, K. Matsuda, and J. Watanabe. Influence of anaesthetic regimens on intestinal absorption in rats. *Pharm. Res.*, **10**:884–8 (1993).
25. D. Winne, H. Görig, and U. Müller. Closed rat jejunal segment *in situ:* role of pre-epithelial diffusion resistance (unstirred layer) in the absorption process and model analysis. *Naunyn-Schm. Arch Pharmacol.*, **335**:204–15 (1987).
26. J. R. Pappenheimer and K. Z. Reiss. Contribution of solvent drag through intercellular junctions to absorption of nutrients by the small intestine of the rat. *J. Membrane Biol.*, **100**:123–36 (1987).
27. U. Fagerholm and H. Lennernäs. Experimental investigation of the effects of transmucosal fluid flow on the jejunal uptake of D_2O and hydrophilic compounds in man and rat. *Pharm. Res.*, **12**:296 (1995).
28. *Clarke's Isolation and Identification of Drugs, 2nd edition.* Ed. A. C. Moffat. The Pharmaceutical Press, London, 1986.
29. *Therapeutic Drugs.* Ed. Sir C. Dollery. Churchill Livingstone, New York, 1991.

30. R. F. Rekker and R. Mannhold. In *Calculation of Drug Lipophilicity.* VCH Publishers, Weinheim, New York, Basel, Cambridge, 1992.
31. T. Itoh, R. Magavi, R. L. Casady, T. Nishihata, and J. H. Rytting. A method to predict percutaneous permeability of various compounds: Shed snake skin as a model membrane. *Pharm. Res.,* 7:1302–6 (1990).
32. A. Sandberg. *Extended-release metoprolol. Dissertation thesis,* Uppsala, 1994.
33. 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**:880–5 (1991).
34. *Martindale, 27th edition.* Ed. A. Wade, The Pharmaceutical Press, London, 1977.
35. G. Paintaud. *Kinetics of drug absorption and influence of absorption rate on pharmacological effect. Dissertation thesis,* Stockholm, 1993.