MODELING OF INTESTINAL DRUG ABSORPTION: ROLES OF TRANSPORTERS AND METABOLIC ENZYMES (FOR THE GILLETTE REVIEW SERIES)

K. SANDY PANG

Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada

This article is available online at http://dmd.aspetjournals.org

Oral Drug Absorption

The absorption of drugs via the oral route is a subject of intense and continuous investigation in the pharmaceutical industry since good bioavailability implies that the drug is able to reach the systemic circulation by mouth. Oral dry absorption is affected by both drug properties and the physiology of the gastrointestinal tract (GIT[1]), or patient properties, including drug dissolution from the dosage form, the manner in which drug interacts with the aqueous environment and membrane, permeation across membrane, and irreversible removal by first-pass organs such as the intestine, liver, and lung (Martinez and Amidon, 2002). The purpose of this minireview is to highlight the processes governing drug bioavailability when the drug is already in solution, and emphasizes the roles of intestinal transporters and metabolism on oral bioavailability. The description of physical models for drug dissolution on drug absorption (Higuchi, 1967) or hepatic modeling (Pang and Chiba, 1994; Pang et al., 1998; Abu-Zahra and Pang, 2000), however, is beyond the scope of this work.

The intestine, in addition to the liver, is an important tissue that regulates the extent of absorption of orally administered drugs, since the intestine and liver are involved in first-pass removal (Gibaldi et al., 1971; Rowland, 1972). The majority of drug absorption occurs at the small intestine because of the large surface area since the presence of villi and microvilli increases the absorptive area manyfold. The duodenum and jejunum possess the greatest surface areas due to the highest concentration of villi and microvilli in these regions, and surface area is least for the ileum (Magee and Dalley, 1986).

The circulation of the intestine is unique in that the intestine is the anterior or portal tissue that regulates the flow of substrates to the liver. The intestinal venous blood constitutes the majority of the blood supply to the liver, accounting for 75% of total liver blood flow. For drugs that are highly cleared by the intestine, the contribution of the liver or lung to drug metabolism will become reduced, whereas for drugs that are poorly extracted by the intestine, the substrate is able to reach the next organs, the liver and the lung, for removal (Gugler et al., 1975; Xu et al., 1989; Hirayama et al., 1990). The concentration of drug entering the intestine (Xu et al., 1989; Hirayama and Pang, 1990) and the intestinal flow rate (Chen and Pang, 1997) alter the rate of drug delivery and affect the degree of saturability of intestinal enzymes. These ultimately affect the rates of intestinal and hepatic first-pass metabolism. Additionally, variables such as other drugs or food that alter the transit times within the gastrointestinal tract further modulate the absorption of substrates by the intestine (Welling, 1984; Kimura and Higaki, 2002).

Drug Properties

Many efforts exist to interrelate the physicochemical properties of the drug with absorption. The drug, whether a weak acid or a weak base, and its pK_a determine the extent of ionization according to the pH partition hypothesis at various pH values (pH 1.3 for stomach and 6 for intestine) of the gastrointestinal tract (Hogben et al., 1957, 1959; Schanker et al., 1957a,b). Deviations from the predictions were found, and these had been explained by the presence of an unstirred water layer (USWL) (Suzuki et al., 1970a,b), or a microclimate pH (Winne, 1977). The concept of absorption potential was then utilized to describe drug absorbability based on the partition coefficient, the solubility, dose, and fraction un-ionized (Dressman et al., 1985; Macheras and Symillides, 1989; Yu et al., 1996). Drugs that are un-ionized or that undergo hydrogen bonding exhibit a much greater lipophilicity toward membrane permeation than their ionic counterparts. Too many hydrogen donor or acceptor groups, however, is not good (Lipinski et al., 1997). Lipophilicity, a major determinant for predicting the extent of membrane permeation, is often correlated with the partition coefficient, when aqueous solubility is not exceeded and when the unstirred water layer is not an imposing barrier (Ungell et al., 1998). For very lipophilic agents, absorption may be rate-limited by the inability of drug traversing the USWL. For very hydrophilic or polar agents, the converse is true, such that membrane resistance is higher than the aqueous layer resistance. However, when drugs possess both hydrophilic and lipophilic qualities and permeate the USWL and membrane well, blood perfusion rate becomes the overall rate-limiting step for absorption. Predictive models based on Lipinski's Rule of Five (Lipinski et al., 1997), the quantitative structure-bioavailability relationship (Andrews et al., 2000), or other quantitative structure-activity relationship models (Norinder et al., 1999; Zhao et al., 2001, 2002; Klopman et al., 2002) have been developed to forecast the drug permeation potentials. The Rule of Five predicts that oral availability will be poor when there are more than 5 H-bond donors, 10 H-bond

Address correspondence to: Dr. K. S. Pang, Faculty of Pharmacy, University of Toronto, 19 Russell Street, Toronto, Ontario Canada M5S 2S2. E-mail: ks.pang@utoronto.ca

acceptors, the logP value is greater than 5, and the molecular weight exceeds 500 (Lipinski et al., 1997).

Drug permeation across the intestinal membrane is described by the effective permeability coefficient, P_{eff}, or the overall rate of loss of compound per unit area (Elliott et al., 1980; Amidon et al., 1981; Ho et al., 1983; Fagerholm et al., 1996). The coefficient P_{eff} may be estimated from luminal perfusion studies by measurement of drug loss across the lumen of any given segment, where $C_{out,lumen}$ and $C_{in,lumen}$ are the concentrations of drug leaving and entering the lumen, respectively; r is the radius, l is the length, and Q_{lumen} is the experimentally determined luminal flow rate.

$$P_{eff} = \frac{Q_{lumen}}{2\pi rl} \ln\left(\frac{C_{out,lumen}}{C_{in,lumen}}\right) \qquad (1)$$

Since changes in water content in the lumen occur for these studies, the change in water volume may be corrected for by the inclusion of a nonabsorbable substance such as polyethylene glycol 4000. For passively absorbed drugs, P_{eff} is dependent on the physicochemical properties of the substrate, including lipophilicity, molecular size, hydrogen bonding capacity, and polar surface area (Winiwarter et al., 1998), and is related to the permeability in the aqueous unstirred water layer (P_{aq}) and the permeability across the membrane (P_m), and may be estimated from the following equation (Ho et al., 1983).

$$\frac{1}{P_{eff}} = \frac{1}{P_{aq}} + \frac{1}{P_m} \qquad (2)$$

The fraction of dose absorbed (F_a or $C_{ss,out,lumen}/C_{ss,in,lumen}$) is estimated as that portion of the dose which disappeared from the intestinal lumen. The fraction of dose absorbed will depend on the physicochemical properties of the compound, the presence of carrier-mediated systems for absorption and exsorption, and intestinal metabolism within the enterocytes.

The Intestine, a Drug Metabolizing and Excretion Tissue

The intestine is noted for its absorptive function because of the presence of villi and microvilli and transporters for organic anions and cations (Fei et al., 1994; Sadee et al., 1995; Tsuji and Tamai, 1996; Arimori and Nakano, 1998; Craddock et al., 1998; Koepsell 1998; Ito et al., 1998; Zhang et al., 1998). Additionally, there exist drug-metabolizing enzymes for oxidation due to the appreciable cytochrome P450 3A (CYP3A4 in humans) (Hoensch et al., 1975; Watkins et al., 1987; Dubey and Singh, 1988a; Kolars et al., 1992; Thummel et al., 1996; Paine et al., 1996, 1997) and conjugation enzymes (Dubey and Singh, 1988b; Ilett et al., 1990), and efflux transporters at the apical and basolateral membranes (Lin et al., 1999; Suzuki and Sugiyama, 2000; Wacher et al., 2001) (Fig. 1). Drug exsorption occurs at the villous tips of the enterocytes at the apical membrane via the 170-kDa P-glycoprotein [Pgp; or the multidrug resistance gene product (MDR1)] (Thiebault et al., 1987; Hsing et al., 1992; Smit et al., 1998a,b) and multidrug resistance-associated protein 2 (MRP2) (Paulusma et al., 1996; Gotoh et al., 2000; Mottino et al., 2000) that cause drug efflux into the lumen, effectively reducing the sojourn of drug within the enterocyte (Saitoh et al., 1996; Lown et al., 1997b; Kim et al., 1998). Many drugs are substrates of both Pgp and cytochrome P450 3A (Terao et al., 1996; Wacher et al., 1998; Benet and Cummins, 2001). These are exemplified by verapamil (Saitoh and Aungst, 1995; Sandström et al., 1998); anticancer drugs such as vincristine, etoposide, daunorubicin, and paclitaxel (Leu and Huang, 1995; Sonnichsen et al., 1995; Nakayama et al., 2000; Chico et al., 2001; Wacher et al., 2001; Abraham et al., 2002); digoxin (Cavet et al., 1996; Greiner et al., 1999); indinavir, the human immunodefi-
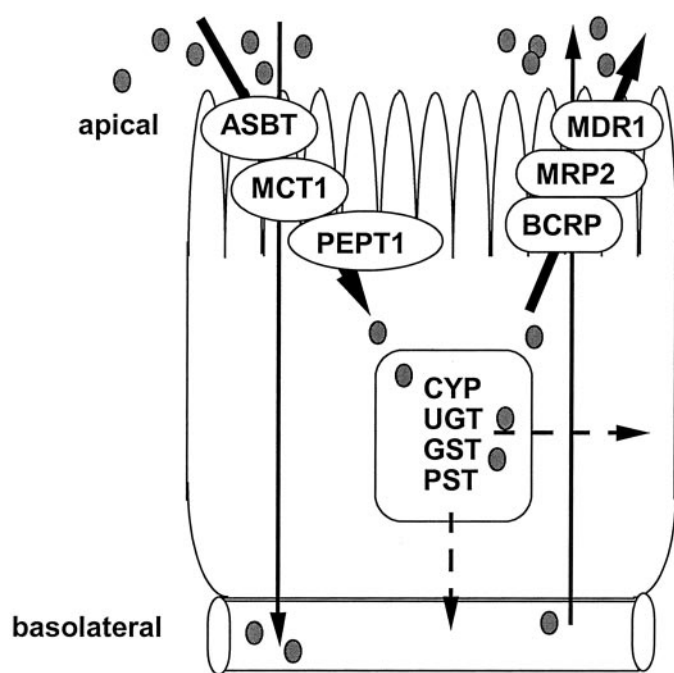

FIG. 1. *Schematic diagram of the enterocyte of the intestine showing absorptive and efflux transporters at the apical and basolateral membranes, and enzymes for intracellular metabolism.*

ciency virus protease inhibitor (Hochman et al., 2000, 2001; Li et al., 2002); and immunosuppressive agents cyclosporin (Gan et al., 1996; Lown et al., 1997b), tacrolimus (Lampen et al., 1995; Hashimoto et al., 1998; Hashida et al., 2001), and sirolimus (Lampen et al., 1998; Paine et al., 2002).

Gastric Emptying and Intestinal Motility

The rate of gastric emptying strongly impacts the rate and extent of intestinal drug metabolism and drug absorption. Various disease conditions and food intake affect stomach emptying and/or intestinal transit (Welling, 1984). Double peak absorption has been correlated with antral gastric motility (Oberle and Amidon,1987; Plusquellec et al., 1987; Suttle et al., 1992; Langguth et al., 1994; Lipka et al., 1995; Suttle and Brouwer, 1995; Wang et al., 1999; Takamatsu et al., 2002; Kimura and Higaki, 2002; Yin et al., 2003) as well as other factors including the presence of adjuvants (Basit et al., 2002) or bile salts (Lennernäs and Regardh, 1993). The data showing double peaks during absorption have been modeled as the discontinuous oral absorption model (Witcher and Boudinot, 1996). Inevitably, a delay in stomach emptying reduces the rate of drug absorption since the rate of delivery to the site of absorption, the small intestine, is prolonged (Table 1). With regard to gastric emptying, drugs may be classified as

TABLE 1

Effect of delayed gastric emptying and effect of metabolism and secretion on drug absorption

Drug Class	Rate of Absorption	Extent of Absorption
Acid-labile compounds	↓	↓
Relatively insoluble compounds	↓	↑
Compounds with good water and lipid solubility	↓	↔
Compounds whose absorption is mediated by intestinal transporters	↓	↑
Compounds that are metabolized by intestine		↓
Compounds that are effluxed by intestine		↓

TABLE 2

Involvement of intestinal transporters for drug absorption (see also references contained in Tsuji and Tamai, 1996)

Transporter	Substrate	Reference
abst or ibat Ileal sodium-dependent bile acid transporter	Taurocholate	Schneider et al. 1995; Craddock et al., 1998
Pept1 Oligopeptide transporter 1	Aminocephalosporins, β-amino lactam antibiotics (cyclacillin, cephalexin, cephradine), acetylcholinesterase inhibitors (benazepril, quinapril), bestatin, renin inhibitors	Fei et al., 1994
Mct1 Monocarboxylic acid transporter 1	Lactate and pyruvate transport (simple aryl and alkyl acids)	
Oatp3 Intestinal organic anion transporting polypeptide 3	Taurocholate, estrone sulfate	Walters et al., 2000
NPT1 Sodium-dependent phosphate tranporter 1	Fosfomycin, foscarnet	Tsuji and Tamai, 1989
OCT1 Organic cation transporter 1	Spermine, spermidine	
Folic acid transporter	Methotrexate	
CNT Nucleoside transporters N1 (purine) and N2 (pyrimidine)	Zidovudine, stavudine, thymidine, ribavirin, allopurinol, 5-fluorouracil	
Amino acid carrier	D- and L-Methionine, *l*-methyldopa, baclofen, taurine, alanine	
MDR1 or Pgp Multidrug resistance protein 1 or P-glycoprotein	Daunomycin, rhodamine 123, verapamil, diltiazem, colchicine, digoxin, quinidine, vincristine, etoposide β-blockers (sotalol, pafenolol, celiprolol, tanilol)	
Mrp2 Multidrug resistance-associated protein 2	Glutathione conjugates, glucuronide conjugates, leukotriene C4, vincristine	Gotoh et al., 2000; Mottino et al., 2000
BCRP Breast cancer resistance protein	Topotecan, mitoxantrone, anthracyclines	Doyle et al., 1998; Zamber et al., 2003
Mrp3		Rost et al., 2002

1) acid-labile compounds, 1) relatively insoluble compounds, 3) drugs with good water and lipid solubility, and 4) drugs absorbed by carriers. For the acid-labile drugs such as penicillin and ampicillin, a greater degradation occurs with prolongation of stay in the stomach, diminishing the extent of absorption (Terry et al., 1982; Ali, 1985). By contrast, increasing the transit time of relatively insoluble compounds in the stomach favors drug dissolution and improves the extent of the absorption of griseofulvin and phenytoin (Aoyagi et al., 1984; Hamaguchi et al., 1993), whereas no change in extent exists for well absorbed drugs such as acetaminophen and fadrozole, compounds of sound water and lipid solubility (Terry et al., 1982; Choi et al., 1993). For drugs whose transport into the intestine is via apical transporters, however, it is envisaged that the reduced intermittent release of drug from the stomach to the intestine brings about a desaturation of the transporter system, rendering an increase in the extent of drug absorption (Table 1).

Methods to Study Intestinal Transport and Metabolism

Because of the significance of the intestine as an important first-pass organ, high-throughput in vitro systems have been developed to assess the importance of intestinal absorption, metabolism, and excretion for the prediction of drug-drug interactions. Gene expression systems (Smit et al., 1998b; Cvetkovic et al., 1999; Gotoh et al., 2000; Shitara et al., 2002) provide direct information on the involvement of individual transporters or enzymes. Then there are the intestinal membrane segments/preparations (Wilson and Treanor, 1975; Hopfer et al., 1976; Lasker and Rickert, 1978; Johnson et al., 2001), cells (Koster and Noordhoek, 1983; Traber et al., 1991), everted sacs (Munck, 1965; Barr and Riegelman, 1970; Kaplan and Cotler, 1972), and the Ussing chamber (Fiddian-Green and Silen, 1975; Rogers et al., 1987; Lampen et al., 1995). For flux measurements, a donor compartment is used for drug administration and a receiving compartment is used for sampling. With drug given to the mucosal side, sampling allows the examination of drug absorption, metabolism, and efflux as well as entry into the basolateral compartment. Moreover, drug may be given at the serosal compartment to ascertain the net flux from the basolateral side to the mucosal lumen. Drug efflux and exsorption has been studied with vesicles prepared from the basolateral and apical membranes (Weinberg et al., 1986; Tsuji and Tamai, 1989; Bair et al., 1991).

A popular in vitro system is the Caco-2 cell line, derived from human colon carcinoma cells (Hidalgo et al., 1989; Hunter et al., 1990), that contains the Pgp (Hunter et al., 1993a,b). A slight drawback may be the existence of a USWL that may pose as a barrier for lipophilic drug transport (Hidalgo et al., 1991). The development of the Caco-2 cell has greatly facilitated progress and led to the testing of diverse drug classes as Pgp substrates (Burton et al., 1993; Hosoya et al., 1996; Tanaka et al., 1996; Terao et al., 1996). The differentiated Caco-2 cell monolayers were initially used to study drug efflux by the Pgp, or the multidrug resistance gene product MDR1. Involvement of Pgp is inferred when the basolateral to apical flux (B to A) exceeds that of A to B. It was further found that, upon culture in 1α-25-dihyroxy vitamin D_3 for 2 weeks postconfluence, cytochrome P450 3A activities were up-regulated (Crespi et al., 1996, 2000; Schmiedlin-Ren et al., 1997; Thummel et al., 2001). More recent development involved transfection with the cytochrome P450 gene and stimulation by butyrate (Cummins et al., 2001) to provide the added P450 activity. The incubation system, the donor and receiving compartments separated by the cell monolayer, is an efficient, high-throughput system for examination of whether newly developed pharmaceuticals are substrates of cytochrome P450 3A4 and/or P-glycoprotein such that interactions with other drugs may be predicted.

TABLE 3

Segmental distribution of transporters and enzymes among the intestinal segments

Transporter	Segmental Distribution	References
Asbt	distal intestine > proximal intestine	Schneider et al., 1995; Aldini et al., 1996
Pept1	proximal intestine > distal ileum function: (jejunum > ileum > duodenum)	Fei et al., 1994
Mct1	duodenum < jejunum > ileum	Tamai et al., 1999; Cong et al., 2001
Oatp3	highest in jejunum	Walters et al., 2000
CNT	highest in jejunum	Ngo et al., 2001
CYP3A	duodenum ∼ jejunum > ileum	Paine et al., 1996, 1997; Li et al., 2002
UGT	duodenum ∼ jejunum > ileum	Koster et al., 1985
PST	duodenum ∼ jejunum > ileum	Schwartz and Schwenk, 1974
GST	duodenum ∼ jejunum > ileum	Pinkus et al., 1977
Estrone sulfatase	proximal > distal	Huijghebaer et al., 1984
Mrp2	duodenum ∼ jejunum > ileum	Mottino et al., 2000; Gotoh et al., 2000
Mdr1	jejunum ∼ ileum > duodenum	Saitoh and Aungst, 1995; Collett et al., 1999; Li et al., 2002
Mrp3 (basolateral)	ileum > jejunum > duodenum	Rost et al., 2002

TABLE 4

Route-dependent intestinal metabolism

Drugs	Route of Administration		References
	Intraduodenal	Systemic	
Acetaminophen	Glucuronide/Sulfate	ND	Pang et al., 1986
Enalapril	Enalaprilat	ND	Pang et al., 1985
Cyclosporin	+	ND	Ducharme et al., 1995
Tacrolimus	+	ND	Lampen et al., 1995
Midazolam	1-Hydroxymidazolam	less	Paine et al., 1996; Thummel et al., 1996
(−)Aminocarbovir	(−)Carbovir	less	Wen et al., 1999
Morphine	Morphine 3β-D-glucuronide	ND	Doherty et al., 2000

The in situ vascularly perfused rat small intestine preparation is a useful preparation for studying the disposition of both orally and systemically administered agents (Windmueller and Spaeth, 1977, 1981; Pang et al., 1986; Hirayama et al., 1989; Doherty and Pang, 2000). In this preparation, the native architecture of the small intestine is maintained with respect to the circulation such that the extents of metabolism, absorption, and secretion can be studied simultaneously (Pang et al., 1985; Xu et al., 1989; Hirayama and Pang, 1990). The technique allows for single-pass or recirculating experiments involving systemic or luminal drug administration, including luminal administration in closed loops or segments (Pang et al., 1986; Cong et al., 2001). In other instances, the intestine-liver preparation may be used for the study of first-pass metabolism and for examination of the role of the intestine on regulation of hepatic metabolism (Pang et al., 1985; Chen and Pang, 1997).

In vivo techniques exist for the study of intestinal drug absorption. The Doluisio method entails use of an in situ rat gut technique for drug administration into the lumen (Doluisio et al., 1969; Sim and Back, 1988). In some rat preparations, the inflow and outflow of a select segment were monitored for drug disappearance, and arterial blood was sampled and the volume of blood was replenished by transfusion (Barr and Riegelman, 1970). Some studies involve luminal instillation of drug to select or closed segments of rats (duodenum, jejunum, or ileum) (Hirayama et al., 1990; Cong et al., 2001) or humans (Gramatté and Richter, 1994; Gramatté, 1996; Gramatté et al., 1994, 1996). Gene knockout mice (Kim et al., 1998; Smit et al., 1998; Greiner et al., 1999) and mutant animal models (Gotoh et al., 2000) have also been utilized to examine intestinal transport. The surgical manipulation of portacaval transposition allowed the direct assessment of systemic intestinal removal in the absence of the liver (Gugler et al., 1975; Effeney et al., 1982; Lo et al., 1982). The method is almost analogous to the oral and systemic administrations of the test drug, midazolam, to anhepatic patients for the estimation of intestinal drug metabolism (Paine et al., 1996). Through varying sites of drug administration, namely, orally, intraportally, and intravenously, comparison of the area under the curves under first-order conditions yields the available fraction of the intestine and liver (Mistry and Houston, 1985, 1987; Hirayama et al., 1990).

Transporters for Absorption And Efflux at Apical Membrane

Much attention is given to the presence of transporters at the apical or brush-border membrane of the intestine, not only for drug absorption but also for exsorption (Table 2). The various apical transporters for organic anions and cations have been reviewed (Tsuji and Tamai, 1996; Koepsell, 1998; Zhang et al., 1998). The ABC (ATP-binding cassette proteins) efflux transporters, Pgp for lipophilic entities and MRP2 for drug conjugates, are both present on the intestinal membrane. Moreover, the newly characterized breast cancer resistance protein (BCRP) multidrug transporter confers resistance to mitoxantrone, topotecan, the anthracyclines, and related drugs in cell lines (Doyle et al., 1998; Allen et al., 1999; 2002; Miyake et al., 1999; Jonker et al., 2000; Zamber et al., 2003). These efflux transporters are known to delimit drug absorption.

Heterogeneity of Intestinal Transporters. Analogous to that found for the liver, there exists an increasing body of literature on the heterogeneity of intestinal transporters (Table 3). The absorption of salicylate (Ungell et al., 1998), antipyrine (Raoof et al., 1998), acetaminophen (Gramatté and Richter, 1994), griseofulvin (Gramatté, 1996), and (−)-carbovir (Soria and Zimmerman, 1994) was found to be the same among all segments. Metoprolol absorption is the same among segments in humans (Vidon et al., 1985). Preferential absorption in segmental regions has also been noted for the intestinal absorption of ranitidine (Suttle and Brouwer, 1995) and diltiazem (Homsy et al., 1995). In like fashion, the absorption of ranitidine
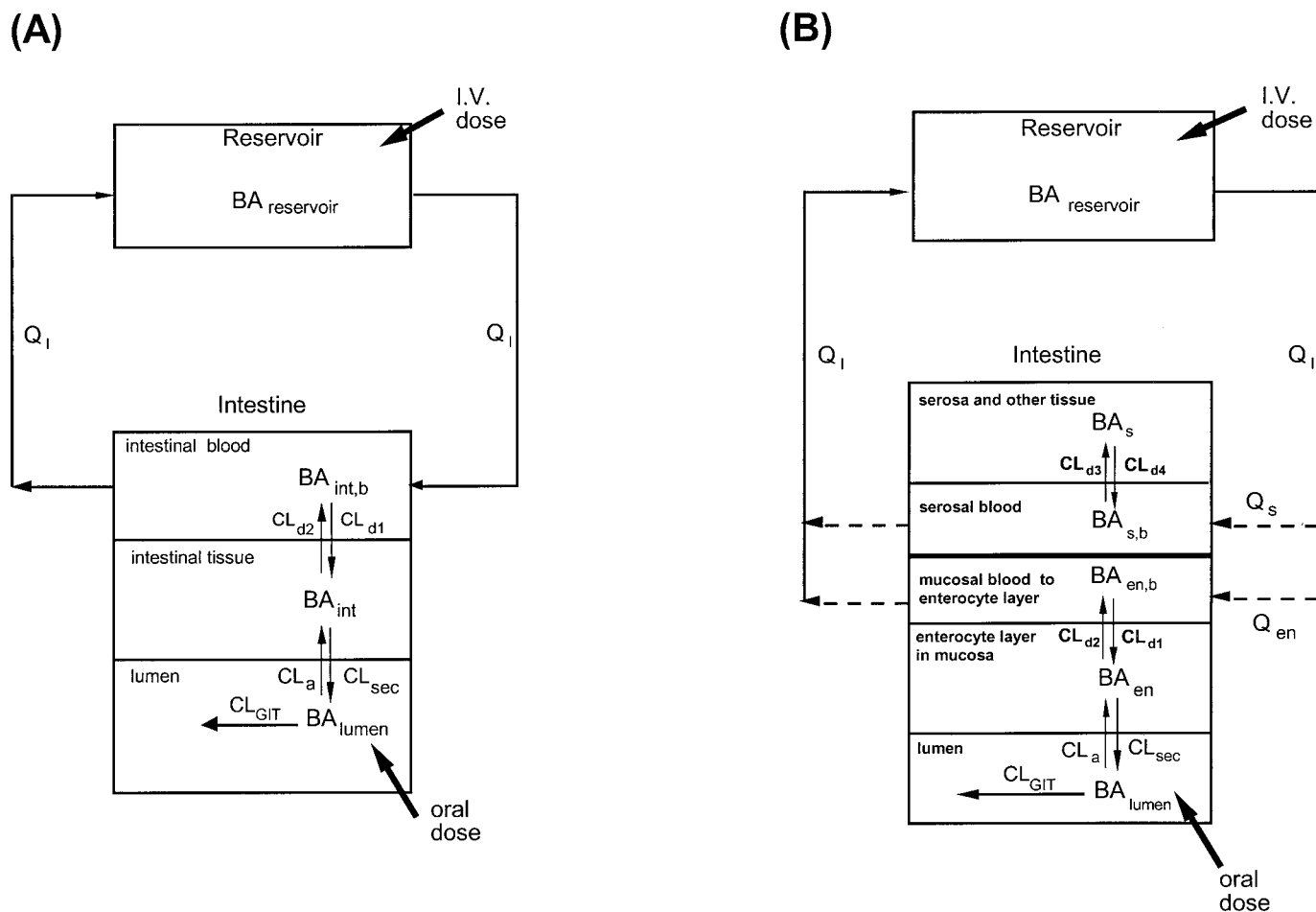
(A)

(B)

FIG. 2. *Fates of drug and metabolite in the TM (A) and SFM (B).*

Note that the entire oral dose passes through the enterocyte region for TM and SFM, whereas only partial intravenous dose reaches the enterocyte region for the SFM. For TM, the intestinal blood (Q_I) perfuses the entire intestinal tissue, the site of metabolism and absorption from the lumen. For SFM, intestinal blood is segregated to perfuse the nonmetabolizing (Q_s) and enterocyte-mucosal (Q_{en}) regions. Drug equilibrates with those in the corresponding tissue layers with intrinsic transfer clearances CL_{d1} and CL_{d2} for TM or CL_{d1} and CL_{d2}, CL_{d3} and CL_{d4} for SFM. The absorptive, metabolic, and efflux activities within the villus tips of the mucosal layer are represented by the rate constant, k_a, and metabolic and secretory intrinsic clearances, $CL_{int,m}$ and $CL_{int,sec}$, respectively. Gastrointestinal transit clearance is denoted by CL_{GIT}. Adapted from Cong et al. (2000), with permission.

(Gramatté et al., 1994) and talinolol (Gramatté et al., 1996) is higher in the proximal intestine in humans. For hydrochlorothiazide, atenolol, furosemide, and cimetidine (Lennernäs, 1998), the net mucosal to serosal absorption was greater for the jejunum than for the ileum. For verapamil (Hunter et al., 1990), phenytoin, almokalant, gemifibrozil, metoprolol, omeprazol, propranolol, foscarnet, erythritol, dDVAP (Ungell et al., 1998), and etoposide (Makhey et al., 1998), net mucosal to serosal absorption was greater for the ileum over the jejunum.

Recent advances in expression cloning of intestinal transporters have provided more definitive tools for the examination of regional distribution of the transporters (Fei et al., 1994; Schneider et al., 1995; Mottino et al., 2000; Walters et al., 2000; Ngo et al., 2001). There are consequences of heterogeneously distributed apical absorptive and secretory transporters. Varying rate constants for benzoic acid intestinal uptake were observed among the segments, and a higher absorption rate constant was shown for the jejunal segment versus the duodenal or ileal segment in the perfused rat intestine preparation. The higher jejunal distribution of Mct1 was confirmed with Western blotting along the length of the rat small intestine (Cong et al., 2001). The proton-coupled oligopeptide transporter of the rabbit was found more abundantly in the proximal intestine (duodenum and jejunum) (Fei et al., 1994); the rat organic anion transporter 3, Oatp3, is higher

in the jejunum (Walters et al., 2000); and the apical bile salt transporter (abst) predominates in the distal ileum of the hamster and rat (Wong et al., 1994; Schneider et al., 1995). Gotoh et al. (2000) demonstrated the greater mRNA expression of Mrp2 in the rat jejunum, followed by the duodenum and ileum, with very little in the colon, as confirmed by Mottino et al. (2000). The excretion of the glutathione conjugate 2,4-dinitrophenyl-S-glutathione by Mrp2 was greatest in the jejunum, as expected by mRNA expression (Gotoh et al., 2000). The efflux transporter rat Mrp2 is higher in the proximal region (Gotoh et al., 2000; Mottino et al., 2000), whereas the Pgp efflux pump is higher distally at the jejunum and ileum (Thiebault et al., 1987; Hunter et al., 1990; Chianale et al., 1995; Lown et al., 1997b; Collett et al., 1999; Nakayama et al., 2000; Stephens et al., 2001; Li et al., 2002). The rat basolateral Mrp3 that transports drug out of the cell is higher toward the distal ileum and colon (Rost et al., 2002). The nonhomogeneous distributions of the transporters (Table 3) are expected to affect drug absorption and bioavailability.

Heterogeneity of Intestinal Enzymes for Metabolism. The intestinal tissue is endowed with phase I and II enzymes, although at lower levels than those for the liver. Drug metabolizing enzymes: UDP-glucuronosyltransferases (UGTs), sulfotransferases (PSTs), and glutathione S-transferases (GSTs) exhibit a decreasing gradient along the

intestinal wall, from duodenum to ileum (Clifton and Kaplowitz, 1977; Pinkus et al., 1977; Schwarz and Schwenk, 1984; Dubey and Singh, 1988a). The human intestinal CYP3A4 shows a slightly lower level at the duodenum before levels rise again at the jejunum, then finally decreasing toward the ileum (Paine et al., 1996; Thummel et al., 1996). The same was found in animals (Hoensch et al., 1975; Li et al., 2002).

Route-Dependent Intestinal Metabolism/Excretion

Route-dependent intestinal metabolism has been observed (Table 4). "Route-dependent" intestinal metabolism describes a greater intestinal metabolism/extraction of drug upon oral or luminal dosing versus "systemic" dosing. In studies pertaining to the perfused, rat small intestine preparations, greater extents of metabolism were noted for acetaminophen (Pang et al., 1986), enalapril (Pang et al., 1985) morphine (Doherty and Pang, 2000) and (−)-aminocarbovir, the prodrug that was converted to (−)-carbovir (Wen et al., 1999), when given luminally, whereas metabolism was either absent or negligible when the drug dose was given into the reservoir for systemic delivery. These results from the vascular intestinal perfusion model mirrored the observations on midazolam hydroxylation in humans. The drug exhibits a low intestinal extraction ratio (0.09) in anhepatic patients undergoing liver transplantation with systemic administration, but extensive first-pass metabolism was noted orally (extraction ratio of 0.43), with much of the intestinally formed primary metabolite, 1-hydroxy midazolam, reaching the hepatic portal blood (Paine et al., 1996; Thummel et al., 1996, 1997). Interestingly, intestinal CYP3A4 levels in humans correlated well with rates of midazolam 1′- and 4-hydroxylation (Thummel et al., 1996) but failed to show a relation with the erythromycin breath test (Lown et al., 1994, 1997b) that is ordinarily used to correlate with liver CYP3A4 levels (Watkins et al., 1989). The exclusivity of the intravenous erythromycin test to liver function only translates to the inaccessibility of the systemically administered substrate to the intestinal mucosal (CYP3A4) enzymes. The overall findings infer that the enzymes for preabsorptive intestinal metabolism are present on enterocytes facing the lumen and are unavailable to drugs in the circulation. A general hypothesis was put forth to explain route-dependent intestinal metabolism: intestinal drug metabolism behaves as if it were a preabsorptive event occurring predominantly during absorption, but little or no intestinal removal occurs for drug in the systemic circulation due to the inaccessibility of enzymes (Doherty and Pang, 1997). Analogously, pretreatment of humans with rifampin, a pregnane X receptor ligand, on Pgp secretion exerted an effect only on the oral but not the intravenous kinetics of digoxin (Greiner et al., 1999), suggesting that the intestinal Pgp is accessible for digoxin administered into the lumen.

Intestinal Modeling

The dynamic interactions of metabolism and secretion, and the role of transporters on drug absorption have been under considerable debate. The thorough understanding of intestinal processes would be learnt through modeling of intestinal data only in the absence of the contribution from the liver. Recent studies on the interactions between the P450s and Pgp in vitro have led to the conclusion that intestinal metabolism is enhanced by the secretory action of P-glycoprotein due to an increase in the mean residence time (MRT) of drug in the intestine (Benet and Cummins, 2001; Cummins et al., 2001; Johnson et al., 2001). Theoretical examinations on Caco-2 cells and intestinal vascular perfusion systems support the notion that the mean residence time was increased, but metabolism under linear conditions was in fact decreased in the presence of secretion, even though the mean residence time has increased; under nonlinear metabolism, instances exist whereby the rate of metabolite accrual may increase, even though the ultimate amount of metabolite formed remained equal to the dose (Tam et al., 2003a). However, under cases of nonlinear metabolism, increased secretion together with rapid re-entry of drug from the apical compartment to the cell evokes increased rates of drug metabolism. The reason for this anomaly is attributed to the desaturation of intestinal enzymes (Tam et al., 2003a).

The next question is, what about the intact intestine? How should data arising from studies pertaining to intestinal metabolism, efflux, absorption, and gastric emptying/intestinal transit be handled? Modeling efforts have included the gastrointestinal absorption and kinetic models that examine absorption of drug from the stomach, duodenum, upper and lower jejunum and ileum, cecum, and large intestine with
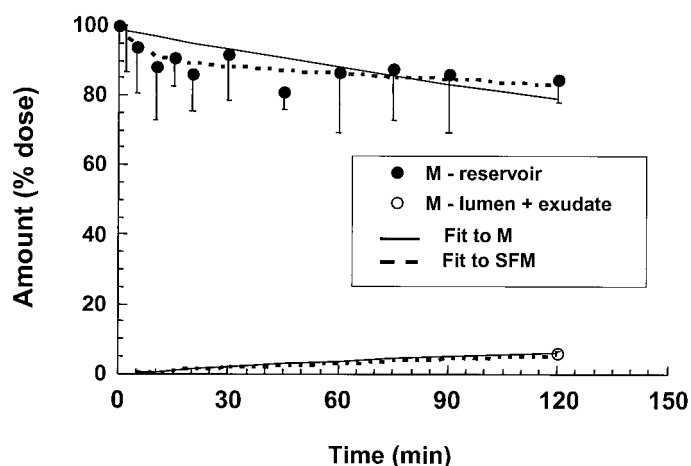
FIG. 3. *Fits of tracer [³H]morphine data from the vascularly perfused, recirculating rat small intestine preparation to the TM and SFM, after systemic administration of dose into the reservoir (data from Cong et al., 2000).*

There was a total lack of morphine glucuronide formed. Only morphine was excreted into the lumen (and luminal fluid that was collected as exudates). Note the superior fit of the data to the SFM. Adapted from Cong et al. (2000), with permission.
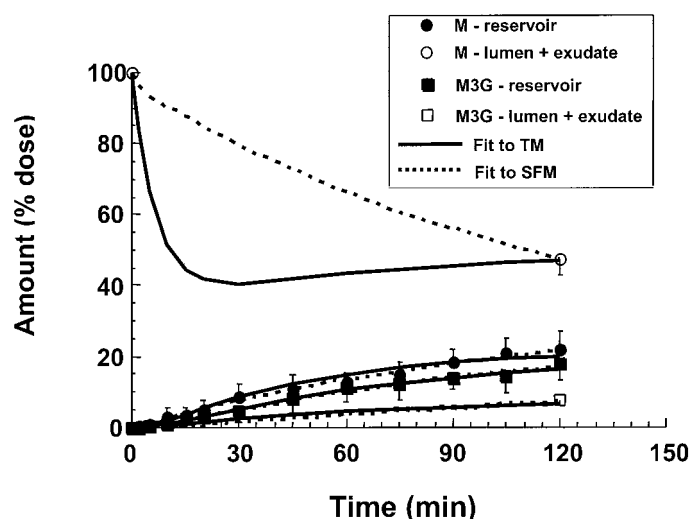
FIG. 4. *Fits of tracer [³H]morphine data from the vascularly perfused, recirculating rat small intestine preparation to the TM and SFM, after duodenal administration of dose into the intestinal lumen (data from Cong et al., 2000).*

Note that morphine glucuronide was formed with luminal administration of morphine. Both morphine and morphine glucuronide were found in the lumen and luminal fluids that were collected as exudates. Note the superior fit of the data to the SFM. Adapted from Cong et al. (2000), with permission.
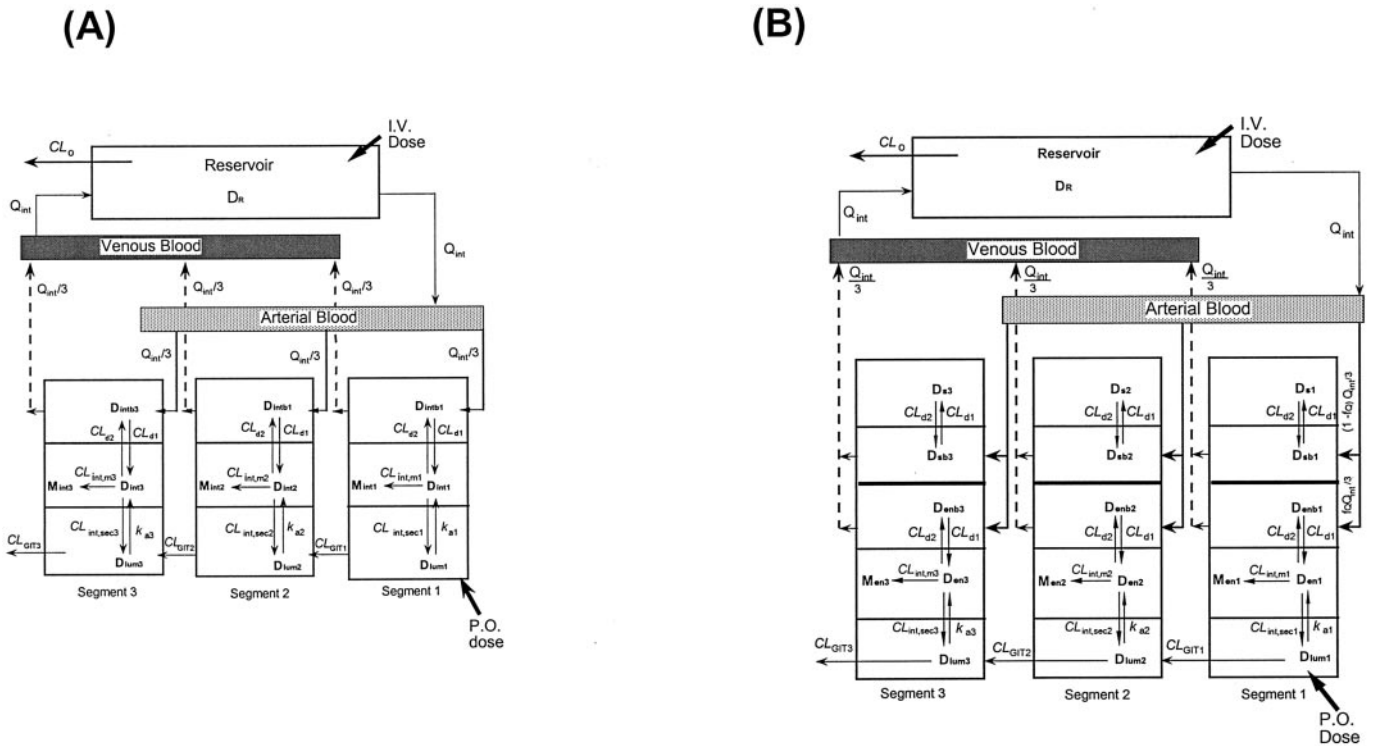
FIG. 5. *The STM, which views the intestine as three equal segments (or expanded i^{th} compartments where i = 1, 2, or 3) of TM (Fig. 2A) with metabolism taking place to form the metabolite (M) in the intestinal tissue (A), and the SSFM, an expansion of the SFM (Fig. 2B), which views the intestine as three segments (or expanded i^{th} compartments where i = 1, 2, or 3) with low and partial flow to the enterocyte regions ($f_Q Q_{int}/3$), where f_Q is the fraction of total intestinal flow (Q_{int}) perfusing the enterocyte region, where the enzymes and efflux transporters reside and bulk flow [($1 - f_Q$) $Q_{int}/3$] to the "other" noneliminating region (B).*

Drug (D) in blood equilibrates with that in the corresponding tissue layers with intrinsic transfer clearances CL_{d1} and CL_{d2} for both STM and SSFM. The absorptive, metabolic, and efflux activities within the villus tips of the mucosal layer are represented by the rate constant, k_{ai}, and metabolic and secretory intrinsic clearances, $CL_{int,mi}$ and $CL_{int,seci}$, respectively, for each ith segment. The gastrointestinal transit clearance is denoted by CL_{GITi}. Adapted from Tam et al. (2003b), with permission.

varying transit times and absorption (Kimura and Higaki, 2002). There had been theoretical investigations on the compartmental absorption and transit (CAT) model that considered the transit and absorption of drug in the small intestine (seven compartments), and there was no absorption within the stomach and colon compartments. Absorption within the seven mixing-tanks in series compartments for the small intestine was governed by the gastric emptying rate and different intestinal transit times (Yu et al., 1996; Yu and Amidon, 1998, 1999). Since the CAT model did not include the physical modeling of drug dissolution from dosage solid forms or controlled release formulations, degradation in lumen, changes in absorptive surface area, absorption in the stomach and colon, metabolism in liver, and transporter densities for absorption and efflux, a refined and expanded model, known as the advanced CAT or ACAT model was developed, with implementation of software, for the prediction of drug absorption (Agoram et al., 2001). Details such as particle size, pH, particle density, and diffusion coefficient were included for consideration of drug dissolution and absorption. In another model, Ito et al. (1999) described intestinal metabolism and secretion, intracellular drug diffusion, and permeation through the basolateral membrane; however, the model seemed to lack description of intestinal flow. Moreover, these models do not relate to blood flow, drug partitioning, and the phenomenon of route-dependent intestinal metabolism. A greater understanding of the interplay will allow sound interpretations on intestinal drug metabolism and transport with respect to other drugs or the intake of fruit juices (Bailey et al., 1993; Lown et al., 1997a; Dresser et al., 2002).

Physiological Models: Traditional Model (TM) and Segregated Flow Model (SFM). Thus, the strategy is to turn to physiologically

based models that encompass all of the salient variables of transport, metabolism, efflux, gastrointestinal transit, and absorption (Doherty and Pang, 2000) (Fig. 2). The TM (Fig. 2A) was developed to describe data arising from recirculation of tracer morphine in the perfused rat small intestine preparation (Doherty and Pang, 2000). In this prepa-
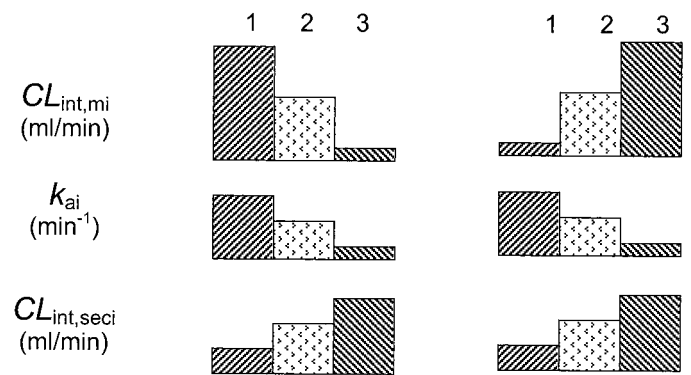

FIG. 6. *Simulations based on intestinal modeling for the rat small intestine, to determine the varying distributions of absorptive and secretory transporters and metabolic enzymes that evoke high and low bioavailabilities.*

Segmental distributions (designated as i^{th} segments where i = 1, 2, and 3) of the metabolic intrinsic clearance, $CL_{int,mi}$ (0.9, 0.5, and 0.1 ml/min), the absorption rate constant, k_{ai} (5, 3, and 1 min^{-1}), and the secretory intrinsic clearance, $CL_{int,seci}$ (2, 4, and 6 ml/min) furnished the lowest bioavailability (F) (left panel) and the highest F (right panel; $CL_{int,seci}$ values were 2, 4, and 6 ml/min; $CL_{int,mi}$ values were 0.1, 0.5, and 0.9 ml/min, and k_{ai} values were 5, 3, and 1 min^{-1}) according to the predictions of the STM and SSFM. The predicted difference existed in the descending versus ascending, segmental distributions of the metabolic intrinsic clearance, $CL_{int,mi}$ within the segments 1, 2, and 3. Taken from Tam et al. (2003b), with permission.

ration, morphine glucuronidation was observed with dosing of morphine into the duodenal lumen and not with administration into the reservoir that mimicked intravenous administration (Figs. 3 and 4). To address the apparent "inaccessibility" of the intestinal enzymes to the drug borne in the circulation but not lumen, or route-dependent metabolism, the segregated flow model, based on modifications of the model of Klippert and Noordhoek (1985) (Fig. 2B), was developed. SFM describes a partial and low blood flow to the "active" enterocyte region where the absorptive and exsorptive carriers and metabolic enzymes reside, and a much higher bulk flow to a nonabsorptive and nonmetabolizing region that included the serosa, submucosa, and mucosa, excluding the enterocyte region (Cong et al., 2000). Thus, the SFM recognizes the subtle demarcation of tissue layers and distributions in blood supply (Svanvik, 1973; Granger et al., 1980). The literature values for the blood flow to the absorptive enterocyte layer

of the mucosa vary greatly, ranging from 5% to 30% (Micflikier et al., 1976; MacFerran and Mailman, 1977; Mailman 1978; Granger et al., 1980). Drug in the lumen must enter via the enterocyte region before reaching the circulation, whereas drug already in circulation is primarily channeled to other tissular regions. The condition conduces to a greater intestinal metabolism with oral versus intravenous dosing. For data on tracer morphine glucuronidation in the recirculating, vascular perfused rat small intestine, the SFM was superior to describe the phenomenon of route-dependent intestinal metabolism (Cong et al., 2000).

These physiological-based models developed for the intestine provide estimations of metabolism and MRT. Under linear conditions, intestinal secretion results in reduced intestinal metabolite formation, as confirmed in a recent theoretical examination of TM and SFM (unpublished data of K. S. Pang, E. Tseng, and D. Tam). The con-

FIG. 7. *Simulations performed for the STM (A) and SSFM (B) to demonstrate the effect of the segmental, gastrointestinal transit clearance (CL_{GITi} = 0.01 or 0.1 ml/min), the secretory intrinsic clearance ($CL_{int,seci}$), and the absorption rate constant (k_{ai}) on the intestinal (systemic) bioavailability, F.*

The metabolic intrinsic clearance, $CL_{int,mi}$, was kept at 0.1 ml/min; the partitioning clearance of drugs, CL_{d1} and CL_{d2}, was 0.9 ml/min. All the intestinal segments (i = 1, 2, and 3) were assigned to be equal and contained equal amounts of transporter, metabolic, and absorptive activities. Adapted from Tam et al. (2003), with permission.
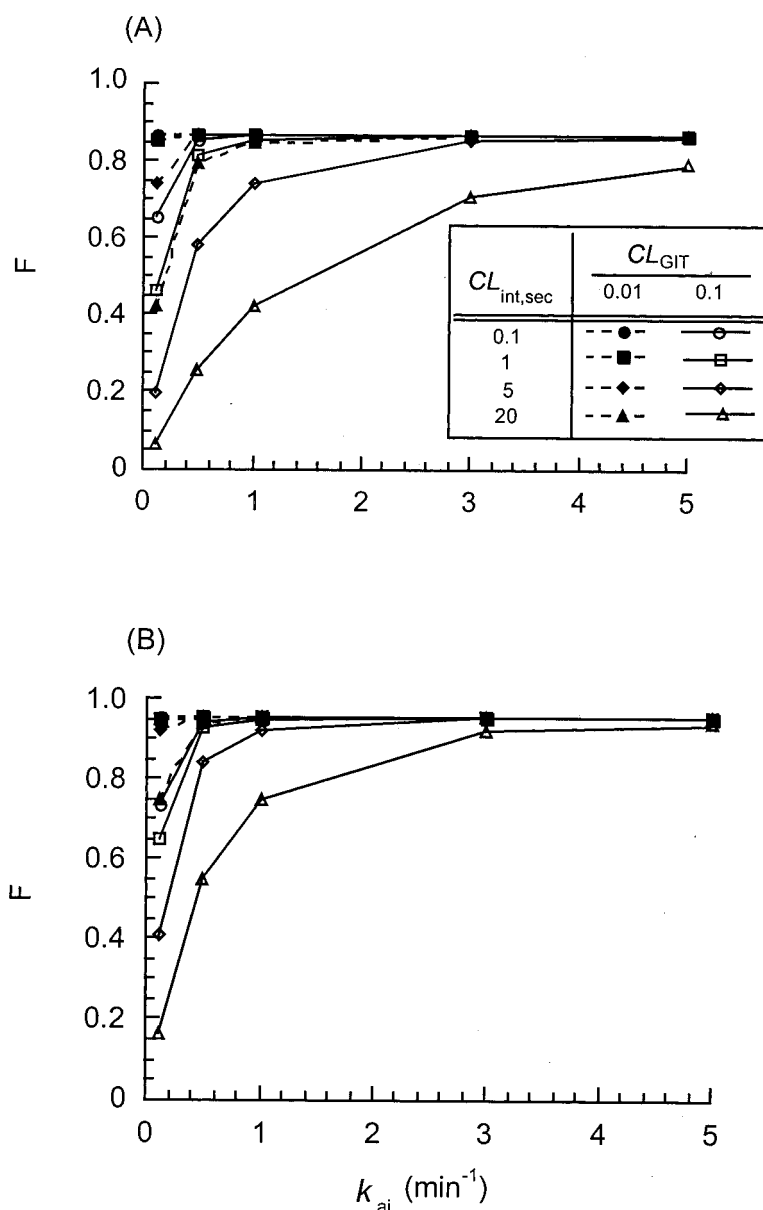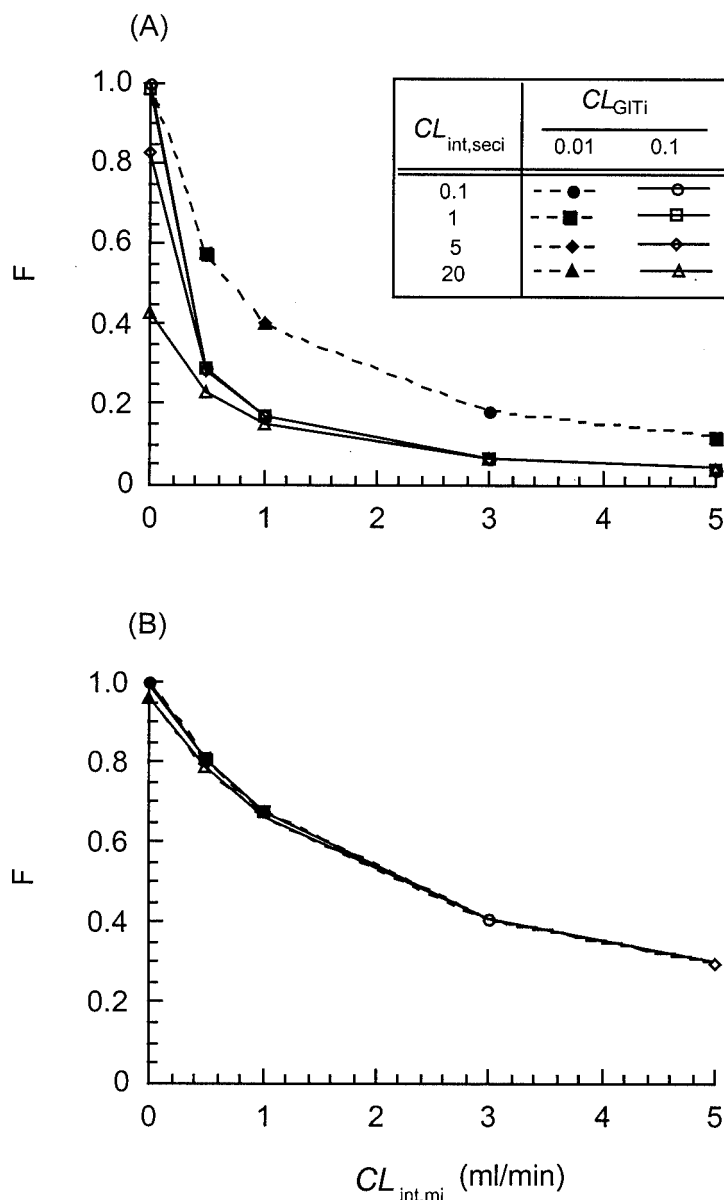
FIG. 8. *Simulations performed for the STM (A) and SSFM (B) to demonstrate the effect of the gastrointestinal transit clearance (CL_{GITi} = 0.01 or 0.1 ml/min), the secretory intrinsic clearance ($CL_{int,seci}$), and the metabolic intrinsic clearance ($CL_{int,mi}$) on the intestinal (systemic) bioavailability, F.*

The absorption rate constant for the each segment, k_{ai}, was kept at 3 min^{-1}; the partitioning clearance for drugs, CL_{d1} and CL_{d2}, was 0.9 ml/min. All the intestinal segments (i = 1, 2, and 3) were assigned to be equal and contained equal amounts of transporter, metabolic, and absorptive activities. Adapted from Tam et al. (2003), with permission.

clusion also agrees with intuitive and deductive reasoning since re- duction of intracellular substrate concentration in the intestine accom- panies drug efflux at the apical membrane, yielding lower rates of intestinal metabolism (Sirianni and Pang, 1997; Schuetz and Schinkel, 1999). This was also found in a simulation study based on Caco-2 cell efflux and metabolism (Tam et al., 2003a). Increased absorption neutralizes the effect of intestinal secretion and increased bioavail- ability (F), whereas increased secretion and metabolism reduce the bioavailability (Table 1).

Segmental, Traditional Model (STM) and Segmental Segre- gated Flow Model (SSFM). To accommodate the attendant hetero- geneities of metabolic and transporter activities and to examine their impact on intestinal clearance and availability, more advanced models have been developed (Tam et al., 2003b). The single intestinal com- partments of the TM and SFM were expanded into three equal

segmental compartments, as for the zonal modeling of the liver (Abu-Zahra and Pang, 2000), in the development of the segmental, traditional model (Fig. 5A) and the segmental, segregated flow model (Fig. 5B) (Tam et al., 2003b). The varying distributions of absorptive and secretory transporters and metabolic enzymes that evoked high and low availabilities were found from simulations based on intestinal modeling for the rat small intestine (Fig. 6). Of note is the strong influence of the distribution of the metabolic enzymes along the intestinal length on F. The predicted trends were generally similar for both STM and SSFM, although the magnitudes differed. When seg- ments of equal activities for the transporters and metabolic enzymes were examined in a simulation study that viewed the intestine as the only eliminating tissue, a fast absorption, reflected by high absorption rate constants within the segments, counteracted the depth of the virtual, peripheral compartment and reduced the mean residence time

of drug in intestinal tissue, MRT_{cell}. Rapid reabsorption of the secreted species canceled the effect of intestinal secretion and increased the F. However, high metabolic and secretion activities within the enterocytes greatly reduced F. A greater metabolic intrinsic clearance within the segments decreased MRT_{cell}, whereas a greater secretory intrinsic clearance within the segments increased the MRT_{cell} (Figs. 7 and 8) (Tam et al., 2003b).

Concluding Remarks and Future Modeling

Another question that remains is whether in vitro data would reflect intestinal metabolism in vivo. But data interpretation and correlation between in vitro and in vivo intestinal drug metabolism/removal is not straightforward. In addition to many of the usual problems often encountered for correlating in vitro-in vivo data on drug metabolism (Pang and Chiba, 1994), other variables are present, and these may further complicate the relationship. One reason is the efflux transporters at the apical membrane and the presence of intestinal motility that work in unison to remove drug out to the lumen, thereby preventing metabolism, although drug may be re-exposed to the enzymes upon reabsorption. Thus, not all of the effluxed drug is subject to intestinal metabolism, even though absorption is rapid. The second is route-dependent intestinal metabolism due to the peculiarity in intestinal blood flow to the "active" metabolic and absorptive region (Cong et al., 2000). The overall estimate of bioavailability is, therefore, complex, and is the consequence of membrane permeation by different arrays of transporters operating in the same or opposite directions, and cellular enzymatic activities that reduce the intracellular drug concentration.

The newly proposed physiologically based models that emphasize metabolism and secretion by the enterocytes have engendered many of the physiological processes governing the overall drug bioavailability and absorption. These models have provided tremendous insight into route-dependent intestinal metabolism/excretion, and the dynamic interplay among intestinal transit times, the absorptive carriers, and metabolic enzymes on intestinal clearance and bioavailability in the absence of a contribution by the liver. For application of the conceptual frameworks developed within the SFM and SSFM, the physiological parameters for the flow rate and volume, as well as more appropriate intestinal transit times, need to be properly scaled-up for modeling of human absorption.

The models, however, considered only drug already in solution. The complexities of drug dissolution and the physiochemical attributes of the various classes of drugs have yet to be integrated to the models. Mass balance with respect to ionization and absorption of the nonionic species may be further accommodated (Ishizaki et al., 1997). The disparate surface area in drug absorption (Agoram et al., 2001) and the intermittent release of drugs in various aggregated and de-aggregated forms from the stomach, gastric emptying, bile salt effects, and the presence of mucus may need to be considered. Improvement in prediction of systemic availability will result upon coupling of the drug-release phase of the ACAT model (Agoram et al., 2001). Sloughed off enterocytes that contribute to intestinal drug metabolism in lumen may need to be modeled (Glaeser et al., 2002). Nonlinearity issues, in terms of intestinal drug-absorptive transporters, metabolic enzymes, and drug efflux transporters, must be considered in drug absorption, especially when the drug is absorbed rapidly to evoke saturation (Tamai et al., 1997; Tam et al., 2003a).

The modeling of drug metabolism and transport by the intestine is at the early stages of development. Proof of the model relies not only on drug but also metabolite disposition during both oral and systemic dosing. Needless to say, the liver should be properly considered for first-pass removal and systemic bioavailability. It is expected that the consolidation of physiologically sound intestinal and liver models will greatly improve our predictiveness on drug absorption bioavailability.

References

Abraham J, Bakke S, Rutt A, Meadows B, Merino M, Alexander R, Schrump D, Bartlett D, Choyke P, Robey R, et al. (2002) A phase II trial of combination chemotherapy and surgical resection for the treatment of metastatic adrenocortical carcinoma: continuous infusion doxorubicin, vincristine and etoposide with daily mitotane as a P-glycoprotein antagonist. *Cancer* **94:**2333–2343.

Abu-Zahra TN and Pang KS (2000) Effect of zonal transport and metabolism on hepatic removal: enalapril hydrolysis in zonal, isolated rat hepatocytes in vitro and correlation with perfusion data. *Drug Metab Dispos* **28:**807–813.

Agoram B, Woltosz WS, and Bolger MB (2001) Predicting the impact of physiological and biochemical processes on oral drug absorption. *Adv Drug Delivery Rev* **50:**S41–S67.

Aldini R, Montagnani M, Roda A, Hrelia S, Biagi PL, and Roda E (1996) Intestinal absorption of bile acids in the rabbit. Different transport rates in jejunum and ileum. *Gastroenterology* **110:**459–468.

Ali HM (1985) Reduced ampicillin bioavailability following oral coadministration with chloroquine. *J Antimicrob Chemother* **15:**781–784.

Allen JD, Brinkhuis RF, Wijnholds J, and Schinkel AH (1999) The mouse Bcrp1/Mxr/Abcp gene: amplification and overexpression in cell lines selected for resistance to topotecan, mitoxantrone, or doxorubicin. *Cancer Res* **59:**4237–4241.

Allen JD, van Loevezijn A, Lakhai JM, van der Valk M, van Tellingen O, Reid G, Schellens JH, Koomen GJ, and Schinkel AH (2002) Potent and specific inhibition of the breast cancer resistance protein multidrug transporter in vitro and in mouse intestine by a novel analogue of fumitremorgin C. *Mol Cancer Ther* **1:**417–425.

Amidon GE, Ho NFH, French AB, and Higuchi WI (1981) Predicted absorption with simultaneous bulk fluid flow in the intestinal tract. *J Theor Biol* **89:**2195–2210.

Andrews CW, Bennet L, and Yu LX (2000) Predicting human oral bioavailability of a compound: development of a novel quantitative structural-bioavailabilty relationship. *Pharm Res (NY)* **17:**639–644.

Aoyagi N, Ogata H, Kaniwa N, and Ejima A (1984) Bioavailability of griseofulvin plain tablets in stomach-emptying controlled rabbits and the correlation with bioavailability in humans. *J Pharmacobiodyn* **7:**630–640.

Arimori K and Nakano M (1998) Drug exsorption from blood into the gastrointestinal tract. *Pharm Res (NY)* **15:**371–376.

Bailey DG, Arnold JM, Strong HA, Munoz C, and Spence JD (1993) Effect of grapefruit juice and naringin on nisoldipine pharmacokinetics. *Clin Pharmacol Ther* **54:**589–594.

Bair CH, Tang MJ, and Huang JD (1991) Concentration-dependent exsorption of quinidine in the rat intestine. *J Pharm Pharmacol* **44:**659–662.

Barr WH and Riegelman S (1970) Intestinal drug absorption and metabolism. I. Comparison of methods and models to study physiological factors of in vitro and in vivo intestinal absorption. *J Pharm Sci* **59:**154–163.

Basit AW, Podczeck F, Newton JM, Waddington WA, Ell PJ, and Lacey LF (2002) Influence of polyethylene glycol 400 on the gastrointestinal absorption of ranitidine. *Pharm Res (NY)* **19:**1368–1374.

Benet LZ and Cummins CL (2001) The drug efflux-metabolism alliance: biochemical aspects. *Adv Drug Delivery Rev* **50:**S3–S11.

Burton PS, Conradi RA, Hilgers AR, and Ho NF (1993) Evidence for a polarized efflux system for peptides in the apical membrane of Caco-2 cells. *Biochem Biophys Res Commun* **190:**760–766.

Cavet ME, West M, and Simmons NL (1996) Transport and epithelial secretion of the cardiac glycoside, digoxin, by human intestinal epithelial (Caco-2) cells. *Br J Pharmacol* **118:**1389–1396.

Chen J and Pang KS (1997) Effect of flow on first-pass metabolism of drugs: single pass studies on 4-methylumbelliferone (4MU) conjugation in the serially perfused rat intestine and liver preparations. *J Pharmacol Exp Ther* **280:**24–31.

Chianale J, Vollrath V, Widlandt AM, Miranda S, Gonzalez R, Fresno AM, Quintana C, Gonzalez S, Andrade L, and Guzman S (1995) Differences between nuclear run-off and mRNA levels for multidrug resistance gene expression in the cephalocaudal axis of the mouse intestine. *Biochim Biophys Acta* **1264:**369–376.

Chico I, Kang MH, Bergan R, Abraham J, Bakke S, Meadows B, Rutt A, Robey R, Choyke P, Merino M, et al. (2001) Phase I study of infusional paclitaxel in combination with the P-glycoprotein antagonist PSC 833. *J Clin Oncol* **19:**832–842.

Choi RL, Sun JX, and Kochak GM (1993) The effect of food on the relative bioavailability of fadrozole hydrochloride. *Biopharm Drug Dispos* **14:**779–784.

Clifton G and Kaplowitz N (1977) The glutathione S-transferases of the small intestine in the rat. *Cancer Res* **37:**788–791.

Collett A, Higgs NB, Sims E, Rowland M, and Warhurst G (1999) Modulation of the permeability of H2 receptor antagonists cimetidine and ranitidine by P-glycoprotein in rat intestine and the human colonic cell line Caco-2. *J Pharmacol Exp Ther* **288:**171–178.

Cong D, Doherty M, and Pang KS (2000) A new physiologically-based segregated flow model to explain route-dependent intestinal metabolism. *Drug Metab Dispos* **28:**224–235.

Cong D, Fong AKY, Lee R, and Pang KS (2001) Absorption of benzoic acid (BA) by segmental regions of the in situ perfused rat small intestine. *Drug Metab Dispos* **29:**1539–1547.

Craddock AL, Loe MW, Daniel RW, Kirby LC, Walters HC, Wong MH, and Dawson PA (1998) Expression and transport properties of the human ileal and renal sodium-dependent bile acid transporter. *Am J Physiol* **274:**G157–G169.

Crespi CL, Fox L, Stocker P, Hu M, and Steimel DT (2000) Analysis of drug transport and metabolism in cell monolayer systems that have been modified by cytochrome P450 3A4 cDNA expression. *Eur J Pharm Sci* **12:**63–68.

Crespi CL, Penman BW, and Hu M (1996) Development of Caco-2 cells expressing high levels of cDNA-derived cytochrome P450 3A4. *Pharm Res (NY)* **13:**1635–1641.

Cummins CL, Mangravite LM, and Benet LZ (2001) Characterizing the expression of CYP3A4 and efflux transporters (P-gp, MRP1, MRP2) in CYP3A4-transfected Caco-2 cells after induction with sodium butyrate and the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate. *Pharm Res (NY)* **18:**1102–1109.

Cvetkovic M, Leake B, Fromm MF, Wilkinson GR, and Kim RB (1999) OATP and P-

glycoprotein transporters mediate the cellular uptake and excretion of fexofenadine. *Drug Metab Dispos* **27:**866–871.

Doherty M and Pang KS (1997) The role of the intestine in the absorption and metabolism of drugs. *Drug Chem Toxicol* **20:**329–344.

Doherty M and Pang KS (2000) Route-dependent metabolism of morphine in the vascularly perfused rat small intestine preparation. *Pharm Res (NY)* **17:**290–297.

Doluisio JT, Billups NF, Dittert LW, Sugita ET, and Swintosky JV (1969) Drug Absorption. I. An in situ rat gut technique yielding realistic absorption rates. *J Pharm Sci* **58:**1196–1200.

Doyle LA, Yang W, Abruzzo LV, Krogmann T, Gao Y, Rishi AK, and Ross DD (1998) A multidrug resistance transporter from human MCF-7 breast cancer cells. *Proc Natl Acad Sci USA* **95:**15665–15670.

Dresser GK, Bailey DG, Leake BF, Schwarz UI, Dawson PA, Freeman DJ, and Kim RB (2002) Fruit juices inhibit organic anion transporting polypeptide-mediated drug uptake to decrease the oral availability of fexofenadine. *Clin Pharmacol Ther* **71:**11–20.

Dressman JB, Amidon GL, and Fleisher D (1985) Absorption potential estimating the fraction absorbed for orally administered compounds. *J Pharm Sci* **74:**588–589.

Dubey RK and Singh J (1988a) Localization and characterization of drug-metabolizing enzymes along the villus-crypt surface of the rat small intestine. I. Monooxygenases. *Biochem Pharmacol* **37:**169–176.

Dubey RK and Singh J (1988b) Localization and characterization of drug-metabolizing enzymes along the villus-crypt surface of the rat small intestine. II. Conjugases. *Biochem Pharmacol* **37:**177–184.

Ducharme MP, Warbasse LH, and Edwards DJ (1995) Disposition of intravenous and oral cyclosporine after administration with grapefruit juice. *Clin Pharmacol Ther* **57:**485–491.

Effeney DJ, Pond SM, Lo MW, Silber BM, and Riegelman S (1982) A technique to study hepatic and intestinal drug metabolism separately in the dog. *J Pharmacol Exp Ther* **22:**507–511.

Elliott RL, Amidon GL, and Lightfoot EN (1980) A convective mass transfer model for determining intestinal wall permeabilities: laminal flow in a circular tube. *J Theor Biol* **87:**757–771.

Fagerholm U, Johansson M, and Lennernäs H (1996) Comparison between permeability coefficients in rat and human jejunum. *Pharm Res (NY)* **13:**1336–1342.

Fei YJ, Kanai Y, Nussberger S, Ganapathy V, Leibach FH, Romero MF, Singh SK, Boron WF, and Hediger MA (1994) Expression cloning of a mammalian proton-coupled oligopeptide transporter. *Nature (Lond)* **368:**563–566.

Fiddian-Green RG and Silen W (1975) Mechanisms of disposal of acid and alkali in rabbit duodenum. *Am J Physiol* **229:**1641–1648.

Gan LS, Moseley MA, Khosla B, Augustijns PF, Bradshaw TP, Hendren RW, and Thakker DR (1996) CYP3A-like cytochrome P-450 mediated metabolism and polarized efflux of cyclosporin A in Caco-2 cells. *Drug Metab Dispos* **24:**344–349.

Gibaldi M, Boyes RN, and Feldman S (1971) The influence of first pass effect on availability of drugs. *J Pharm Sci* **60:**1338–1340.

Glaeser H, Drescher S, von der Kuip H, Behrens C, Geick A, Burk O, Dent J, Somogyi A, von Richter O, Griese E-U, et al. (2002) Shed human enterocytes as a tool for the study of expression and function of intestinal drug-metabolizing enzymes and transporters. *Clin Pharmacol Ther* **71:**131–140.

Gotoh Y, Suzuki H, Kinoshita S, Hirohashi T, Kato Y, and Sugiyama Y (2000) Involvement of an organic anion transporter (canalicular multispecific organic anion transporter/multidrug resistance-associated protein 2) in gastrointestinal secretion of glutathione conjugates in rats. *J Pharmacol Exp Ther* **292:**433–439.

Gramatté T (1996) Griseofulvin absorption from different sites in the human small intestine. *Biopharm Drug Dispos* **15:**747–759.

Gramatté T, El Desoky E, and Klotz U (1994) (1994) Site-dependent small intestinal absorption of ranitidine. *Eur J Clin Pharmacol* **46:**253–259.

Gramatté T, Oertel R, Terhaag B, and Kirch W (1996) Direct demonstration of small intestinal secretion and site-dependent absorption of the beta-blocker talinolol in humans. *Clin Pharmacol Ther* **59:**541–549.

Gramatté T and Richter K (1994) Paracetamol absorption from different sites in the human small intestine. *Br J Clin Pharmacol* **37:**608–611.

Granger DN, Richardson DI, Kvietys PR, and Mortillard NA (1980) Intestinal blood flow. *Gastroenterology* **78:**837–863.

Greiner B, Eichelbaum M, Fritz P, Kreichgauer HP, von Richter O, Zundler J, and Kroemer HK (1999) The role of intestinal P-glycoprotein in the interaction of digoxin and rifampin. *J Clin Investig* **104:**147–153.

Gugler R, Lain P, and Azarnoff DL (1975) Effect of portacaval shunt on the disposition of drugs with and without first-pass effect. *J Pharmacol Exp Ther* **195:**416–423.

Hamaguchi T, Shinkuma D, Irie T, Yamanaka Y, Morita Y, Iwamoto B, Miyoshi K, and Mizuno N (1993) Effect of a high-fat meal on the bioavailability of phenytoin in a commercial powder with a large particle size. *Int J Clin Pharmacol Ther* **31:**326–330.

Hashida T, Masuda S, Uemoto S, Saito H, Tanaka K, and Inui K (2001) Pharmacokinetic and prognostic significance of intestinal MDR1 expression in recipients of living-donor liver transplantation. *Clin Pharmacol Ther* **69:**308–316.

Hashimoto Y, Sasa H, Shimomura M, and Inui K-I (1998) Effects of intestinal and hepatic metabolism on the bioavailability of tacrolimus in rats. *Pharm Res (NY)* **15:**1609–1613.

Hidalgo IJ, Hillgren KM, Grass GM, and Borchardt RT (1991) Characterization of the unstirred water layer in Caco-2 cell monolayers using a novel diffusion apparatus. *Pharm Res (NY)* **8:**222–227.

Hidalgo IJ, Raub TJ, and Borchardt RT (1989) Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. *Gastroenterology* **96:**736–749.

Higuchi WI (1967) Diffusional models useful in biopharmaceutics. Drug release rate processes. *J Pharm Sci* **56:**315–324.

Hirayama H, Morgado J, Gasinska I, and Pang KS (1990) Estimations of intestinal and liver extraction in the *in vivo* rat: studies on gentisamide conjugation. *Drug Metab Dispos* **18:**580–587.

Hirayama H and Pang KS (1990) First-pass metabolism of gentisamide: influence of intestinal metabolism on hepatic formation of conjugates. Studies in the once-through vascularly perfused rat intestine-liver preparation. *Drug Metab Dispos* **18:**580–587.

Hirayama H, Xu X, and Pang KS (1989) Viability of the vascularly perfused, recirculating rat intestine and intestine-liver preparations. *Am J Physiol* **89:**G249–G258.

Ho NFH, Park JY, Ni PF, and Higuchi WI (1983) Advancing quantitative and mechanistic approaches in interfacing gastrointestinal drug absorption studies in animal and humans, in *Animal Models for Oral Drug Delivery in Man: In Situ and In Vivo Approaches* (Crouthamel W and Sarupu A eds) pp 27–106, American Pharmaceutical Association, Washington, D.C.

Hochman JH, Chiba M, Nishime J, Yamazaki M, and Lin JH (2000) Influence of P-glycoprotein on the transport and metabolism of indinavir in Caco-2 cells expressing cytochrome P-450 3A4. *J Pharmacol Exp Ther* **292:**310–318.

Hochman JH, Chiba M, Yamazaki M, Tang C, and Lin JH (2001) P-glycoprotein mediated efflux of indinavir metabolites in Caco-2 cells expressing cytochrome P-450 3A4. *J Pharmacol Exp Ther* **298:**323–330.

Hoensch H, Woo CH, and Schmid R (1975) Cytochrome P450 and drug metabolism in intestinal villous and crypt cells of rats. Effects of dietary iron. *Biochem Biophys Res Commun* **65:**399–406.

Hogben CAM, Tocco DJ, Brodie BB, and Schanker LS (1957) Absorption of drugs from the stomach. II. The human. *J Pharmacol Exp Ther* **120:**540–545.

Hogben CAM, Tocco DJ, Brodie BB, and Schanker LS (1959) On the mechanism of intestinal absorption of drugs. *J Pharmacol Exp Ther* **125:**275–282.

Homsy W, Caille G, and du Souich P (1995) The site of absorption in small intestine determines diltiazem bioavailability in rabbit. *Pharm Res (NY)* **12:**1722–1726.

Hopfer U, Sigrist-Nelson K, and Groseclose R (1976) Jejunal and ileal D-glucose transport in isolated brush border membranes. *Biochim Biophys Acta* **426:**349–353.

Hosoya K-I, Kim K-J, and Lee VHL (1996) Age-dependent expression of P-glycoprotein gp170 in Caco-2 cell monolayers. *Pharm Res (NY)* **13:**885–890.

Hsing S, Gatmaitan Z, and Arias IM (1992) The function of Gp170, the multidrug-resistance gene product, in the brush border of rat intestinal mucosa. *Gastroenterology* **102:**879–885.

Huijghebaer SM, Sim SM, Back DJ, and Eyssen HJ (1984) Distribution of estrone sulfatase activity in the intestine of germfree and conventional rats. *J Steroid Biochem* **20:**1175–1179.

Hunter J, Hirst BH, and Simmons NL (1993a) Drug absorption limited by P-glycoprotein-mediated secretory drug transport in human intestinal epithelial Caco-2 cell layers. *Pharm Res (NY)* **10:**743–749.

Hunter J, Jepson MA, Tsuruo T, Simmons NL, and Hirst BH (1990) Functional expression of P-glycoprotein in apical membranes of human intestinal Caco-2 Cells. *J Biol Chem* **268:**14991–14997.

Hunter J, Jepson MA, Tsuruo, Simmons NL, and Hirst BH (1993b) Functional expression of P-glycoprotein in apical membranes of human intestinal Caco-2 Cells. *J Biol Chem* **268:**14991–14997.

Ilett KF, Tee LB, Reeves PT, and Minchin RF (1990) Metabolism of drugs and other xenobiotics in the gut lumen and wall. *Pharmacol Ther* **46:**67–93.

Ishizaki J, Yokogawa K, Nakashima E, and Ichimura F (1997) Relationships between the hepatic intrinsic clearance or cell-plasma partition coefficient in the rabbit and lipophilicity of basic drugs. *J Pharm Pharmacol* **49:**768–772.

Ito K, Iwatsubo T, Kanamitsu S, Nakajima Y, and Sugiyama Y (1998) Quantitative prediction of in vivo drug clearance and drug interactions from in vitro data on metabolism, together with binding and transport. *Annu Rev Pharmacol Toxicol* **38:**461–499.

Ito K, Kusuhara H, and Sugiyama Y (1999) Effects of intestinal CYP3A4 and P-glycoprotein on oral drug absorption—theoretical approach. *Pharm Res (NY)* **16:**225–231.

Johnson BM, Charman WN, and Porter CJH (2001) The impact of P-glycoprotein efflux on enterocyte residence time and enterocyte-based metabolism of verapamil. *J Pharm Pharmacol* **53:**1611–1619.

Jonker JW, Smit JW, Brinkhuis F, Maliepaard M, Beijnen JH, Schellens JHM, and Schinkel AH (2000) Role of breast cancer resistance protein in the bioavailability and fetal penetration of topotecan. *J Natl Cancer Inst* **92:**1651–1656.

Kaplan SA and Cotler S (1972) Use of cannulated everted intestinal sac for serial sampling as a drug absorbability (permeability) screen. *J Pharm Sci* **61:**1361–1365.

Kim RB, Fromm MF, Wandel C, Leake B, Wood AJJ, Roden DM, and Wilkinson GR (1998) The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors. *J Clin Investig* **101:**289–294.

Kimura T and Higaki K (2002) Gastrointestinal transit and drug absorption. *Biol Pharm Bull* **25:**149–164.

Klippert PJ and Noordhoek J (1985) The area under the curve of metabolites for drugs and metabolites cleared by the liver and extrahepatic organs. Its dependence on the administration route of precursor drug. *Drug Metab Dispos* **13:**97–101.

Klopman G, Stefan LR, and Saiakhov RD (2002) ADME evaluation. 2. A computer model for the prediction of intestinal absorption in humans. *Eur J Pharm Sci* **17:**253–263.

Koepsell H (1998) Organic cation transporters in intestine, kidney, liver and brain. *Annu Rev Physiol* **60:**243–266.

Kolars JC, Schmiedlin-Ren P, Schuetz JD, Fang C, and Watkins PB (1992) Identification of rifampin-inducible P450IIIA4 (CY3A4) in human small bowel enterocytes. *J Clin Investig* **90:**1871–1878.

Koster AS, Frankhuijzen-Sierevogel AC, and Noordhoek J (1985) Glucuronidation of morphine and six β2-sympathomimetics in isolated rat intestinal epithelial cells. *Drug Metab Dispos* **13:**232–237.

Koster AS and Noordhoek J (1983) Glucuronidation in isolated perfused rat intestinal segments after mucosal and serosal administration of 1-naphthol. *J Pharmacol Exp Ther* **226:**533–538.

Lampen A. Christians W, Guengerich P, Watkins PB, Kolars J, Bader A, Gonschior A-K, Dralle H, Hackbarth I, and Sewing K-F (1995) Metabolism of the immunosuppressant tacrolimus in the small intestine: cytochrome P450, drug interactions and interindividual variability. *Drug Metab Dispos* **23:**1315–1324.

Lampen A, Zhang Y, Hackbarth I, Benet LZ,. Sewing K-F, and Christians U (1998) Metabolism and transport of the macrolide sirolimus in the small intestine. *J Pharmacol Exp Ther* **285:**1104–1112.

Langguth P, Lee KM, Spahn-Langguth H, and Amidon GL (1994) Variable gastric emptying and discontinuities in drug absorption profiles: dependence of rates and extent of cimetidine absorption on motility phase and pH. *Biopharm Drug Dispos* **15:**719–746.

Lasker J and Rickert DE (1978) Absorption and glucuronosylation of diethylstilbestrol by the rat small intestine. *Xenobiotica* **8:**665–672.

Lennernäs H (1998) Human intestinal permeability. *J Pharm Sci* **87:**403–410.

Lennernäs H and Regardh CG (1993) Evidence for an interaction between the beta-blocker pafenolol and bile salts in the intestinal lumen of the rat leading to dose-dependent oral absorption and double peaks in the plasma concentration-time profile. *Pharm Res (NY)* **10:**879–883.

Leu B-L and Huang J-D (1995) Inhibition of intestinal P-glycoprotein and effects on etoposide absorption. *Cancer Chemother Pharmacol* **35:**432–436.

Li LY, Amidon GL, Kim JS, Heimbach T, Kesisoglou F, Topliss JT, and Fleisher D (2002) Intestinal metabolism promotes regional differences in apical uptake of indinavir: coupled effect of P-glycoprotein and cytochrome P450 3A on indinavir membrane permeability. *J Pharmacol Exp Ther* **301:**586–593.

Lin JH, Chiba M, and Baillie TA (1999) Is the role of the small intestine in first-pass metabolism overemphasized? *Pharmacol Rev* **51:**135–158.

Lipinski CA, Lombardo F, Dominy BW, and Feeney PJ (1997) Experimental and computational approaches to estimate solubility and permeability in drug discovery and developmental settings. *Adv Drug Delivery Rev* **46:**3–25.

Lipka E, Lee ID, Langguth P, Spahn-Langguth H, Mutschler E, and Amidon GL (1995) Celiprolol double-peak occurrence and gastric motility: nonlinear mixed effects modeling of bioavailability data obtained in dogs. *J Pharmacokinet Biopharm* **23:**267–286.

Lo MW, Effeney DJ, Pond SM, Silber BM, and Riegelman S (1982) Lack of gastrointestinal metabolism of propranolol in dogs after portacaval transposition. *J Pharmacol Exp Ther* **221:**512–515.

Lown KS, Bailey DG, Fontana RJ, Janardan SK, Adair CH, Fortlage LA, Brown MB, Guo W, and Watkins PB (1997a) Grapefruit juice increases felodipine oral availability in humans by decreasing intestinal protein expression. *J Clin Investig* **99:**2545–2553.

Lown KS, Kolars JC, Thummel KE, Barnett JL, Kune KL, Wrighton SA, and Watkins PB (1994) Interpatient heterogeneity in expression of CYP3A4 and CYP3A5 in small bowel. Lack of prediction by the erythromycin breath test. *Drug Metab Dispos* **22:**947–955.

Lown KS, Mayo RR, Leichtman AB, Hsiao H-L, Turgeon K, Schmiedlin-Ren P, Brown MB, Guo W, Rossi SJ, Benet LZ, and Watkins PB (1997b) Role of intestinal P-glycoprotein (mdr1) in interpatient variation in the oral bioavailability of cyclosporine. *Clin Pharmacol Ther* **62:**48–60.

MacFerran S and Mailman D (1977) Effects of glucagon on canine intestinal sodium and water fluxes and regional blood flow. *J Physiol* **266:**1–12.

Macheras P and Symillides MY (1989) Towards a quantitative approach for the prediction of the fraction of dose absorbed using the absorption potential concept. *Biopharm Drug Dispos* **10:**43–53.

Magee DF and Dalley AF II (1986) *Digestion and The Structure and Function of The Gut* (*Karger Continuing Education Series*, vol. 8). Karger, Basel.

Mailman D (1978) Effects of vasoactive intestinal polypeptide on intestinal absorption and blood flow. *J Physiol* **279:**121–132.

Makhey VD, Guo A, Norris DA, Hu P, Yan J, and Sinko PJ (1998) Characterization of the regional intestinal kinetics of drug efflux in rat and human intestine and in Caco-2 cells. *Pharm Res (NY)* **15:**1160–1167.

Martinez MN and Amidon GL (2002) A mechanistic approach to understanding the factors affecting drug absorption: a review of fundamentals. *J Clin Pharmacol* **42:**620–643.

Micflikier AB, Bond JH, Sircar B, and Levitt MD (1976) Intestinal villous blood flow measured with carbon monoxide and microspheres. *Am J Physiol* **230:**916–919.

Mistry M and Houston JB (1985) Quantitation of extrahepatic metabolism. Pulmonary and intestinal conjugation of naphthol. *Drug Metab Dispos* **13:**740–745.

Mistry M and Houston JB (1987) Glucuronidation in vitro and in vivo. Comparison of intestinal and hepatic conjugation of morphine, naloxone and buprenorphine. *Drug Metab Dispos* **15:**710–717.

Miyake K, Mickley L, Litman T, Zhan Z, Robey R, Cristensen B, Brangi M, Greenberger L, Dean M, Fojo T, and Bates SE (1999) Molecular cloning of cDNAs which are highly overexpressed in mitoxantrone-resistant cells: demonstration of homology to ABC transport genes. *Cancer Res* **59:**8–13.

Mottino AD, Hoffman T, Jennes L, and Vore M (2000) Expression and localization of multidrug resistant protein mrp2 in rat small intestine. *J Pharmacol Exp Ther* **293:**717–723.

Munck BG (1965) Amino acid transport by the small intestine of the rat. The effect of amino acid pre-loading on the trans-intestinal amino acid transport by the everted sac preparation. *Biochim Biophys Acta* **109:**142–150.

Nakayama A, Saitoh H, Oda M, Takada M, and Aungst BJ (2000) Region-dependent disappearance of vinblastine in the rat small intestine and characterization of its P-glycoprotein-mediated efflux system. *Eur J Pharm Sci* **11:**317–324.

Ngo LY, Patil SD, and Unadkat JD (2001) Ontogenic and longitudinal activity of Na+-nucleoside transporters in the human intestine. *Am J Physiol* **280:**G475–G481.

Norinder U, Osterberg T, and Artursson P (1999) Theoretical calculation and prediction of intestinal absorption of drugs in humans using MolSurf parameterization and PLS statistics. *Eur J Pharm Sci* **8:**49–56.

Oberle RL and Amidon GL (1987) The influence of variable gastric emptying and intestinal transit rates on the plasma level curve of cimetidine; an explanation for the double peak phenomenon. *J Pharmacokinet Biopharm* **15:**529–544.

Paine MF, Khalighi M, Fisher JM, Shen DD, Kunze KL, Marsh CL, Perkins JD, and Thummel KE (1997) Characterization of interintestinal and intraintestinal variations in human CYP3A-dependent metabolism. *J Pharmacol Exp Ther* **283:**1552–1562.

Paine MF, Leung LY, Lim HK, Liao K, Oganesian A, Zhang MY, Thummel KE, and Watkins PB (2002) Identification of a novel route of extraction of sirolimus in human small intestine: roles of metabolism and secretion. *J Pharmacol Exp Ther* **301:**174–186.

Paine MF, Shen DD, Kunze KL, Perkins JD, Marsh CL, McVicar JP, Barr DM, Gilles BS, and Thummel KE (1996) First-pass metabolism of midazolam by the human intestine. *Clin Pharmacol Ther* **60:**14–24.

Pang KS, Cherry WF, and Ulm EH (1985) Disposition of enalapril in the perfused rat intestine-liver preparation: absorption, metabolism and first pass effect. *J Pharmacol Exp Ther* **233:**788–795.

Pang KS and Chiba M (1994) Metabolism: scaling up from *in vitro* to organ and whole body, in *Handbook of Experimental Pharmacology* (Welling PG and Balant LP eds) pp 101–187, Springer-Verlag, Stuttgart.

Pang KS, Fayz S, Yuen V, te Koppele JM, and Mulder GJ (1986) Absorption and metabolism of acetaminophen by the *in situ* perfused rat small intestine preparation. *Drug Metab Dispos* **14:**102–113.

Pang KS, Geng W, Schwab AJ, and Goresky CA (1998) Probing the structure and function of the liver with the multiple indicator dilution technique, in *Whole Organ Approach to Cellular Metabolism: Capillary Permeation, Cellular Transport and Reaction Kinetics* (Bassingthwaighte J, Goresky CA, and Lenihan JN eds) pp 325–368, Springer-Verlag, Stuttgart.

Paulusma CC, Bosma PJ, Zaman GJ, Bakker CT, Otter M, Scheffer GL, Scheper RJ, Borst P, and Oude Elferink RP (1996) Congenital jaundice in rats with a mutation in a multidrug resistance-associated protein gene. *Science (Wash DC)* **271:**1126–1128.

Pinkus LM, Ketley JN, and Jakoby WB (1977) The glutathione S-transferases as a possible detoxication system of rat intestinal epithelium. *Biochem Pharmacol* **26:**2359–2363.

Plusquellec Y, Campistron G, Staveris S. Barre J, Jung L, Tillement JP, and Houin G (1987) A double peak phenomenon in the pharmacokinetics of veralipride after oral administration: a double-site model for drug absorption. *J Pharmacokinet Biopharm* **15:**225–239.

Raoof AA, Butler J, and Devane JG (1998) Assessment of regional differences in intestinal fluid movement in the rat using a modified in situ single pass perfusion model. *Pharm Res (NY)* **15:**1314–1316.

Rogers SM, Back DJ, and Orme ML (1987) Intestinal metabolism of ethinylestradiol and paracetamol in vitro: studies using Ussing chambers. *Br J Clin Pharmacol* **23:**727–734.

Rost D, Mahner S, Sugiyama Y, and Stremmel W (2002) Expression and localization of the multidrug resistance-associated protein 3 in rat small and large intestine. *Am J Physiol* **282:**G720–G726.

Rowland M (1972) The influence of route of administration on drug availability. *J Pharm Sci* **101:**70–74.

Sadee W, Drubbisch V, and Amidon GL (1995) Biology of membrane transport proteins. *Pharm Res (NY)* **12:**1823–1837.

Saitoh H and Aungst BJ (1995) Possible involvement of multiple P-glycoprotein mediated efflux systems in the transport of verapamil and other organic cations across rat intestine. *Pharm Res (NY)* **12:**1304–1310.

Saitoh H, Gerard C, and Aungst BJ (1996) The secretory intestinal transport of some beta-lactam antibiotics and anionic compounds: a mechanism contributing to poor oral absorption. *J Pharmacol Exp Ther* **278:**205–211.

Sandström R, Karlsson A, and Lennernäs H (1998) The absence of stereoselective P-glycoprotein-mediated transport of R/S-verapamil across the rat jejunum. *J Pharm Pharmacol* **50:**729–735.

Schanker LS, Shore PA, Brodie BB, and Hogben CAM (1957a) Absorption of drugs from the stomach. I. The rat. *J Pharmacol Exp Ther* **120:**528–539.

Schanker LS, Tocco DJ, Brodie BB, and Hogben CAM (1957b) Absorption of drugs from the rat small intestine. *J Pharmacol Exp Ther* **121:**81–88.

Schmiedlin-Ren P, Thummel KE, Fisher JM, Paine MF, Lown KS, and Watkins PB (1997) Expression of enzymatically active CYP3A4 by Caco-2 cells grown on extracellular matrix-coated permeable supports in the presence of 1α-25-dihydroxyvitamin D_3. *Mol Pharmacol* **51:**741–754.

Schneider BL, Dawson PA, Christie DM, Hardikar W, Wong MH, and Suchy FJ (1995) Cloning and molecular characterization of the ontogeny of a rat ileal sodium-dependent bile acid transporter. *J Clin Investig* **95:**745–754.

Schuetz EG and Schinkel AH (1999) Drug disposition as determined by the interplay between drug-transporting and drug-metabolizing systems. *J Biochem Mol Toxicol* **13:**219–222.

Schwarz LR and Schwenk M (1984) Sulfation in isolated enterocytes of guinea pig: dependence of inorganic sulfate. *Biochem Pharmacol* **33:**3353–3356.

Shitara Y, Sugiyama D, Kusuhara H, Kato Y, Abe T, Meier PJ, Itoh T, and Sugiyama Y (2002) Comparative inhibitory effects of different compounds on rat oatp1 (slc21a1)- and Oatp2 (Slc21a5)-mediated transport. *Pharm Res (NY)* **19:**147–153.

Sim SM and Back DJ (1986) Intestinal absorption of oestrone, oestrone glucuronide and oestrone sulphate in the rat in situ. II. Studies with the Doluisio technique. *J Steroid Biochem* **24:**1085–1089.

Sirianni GL and Pang KS (1997) Organ clearance concepts: new perspectives on old principles. *J Pharmacokinet Biopharm* **25:**457–478.

Smit JW, Schinkel AH, Müller M, Weert B, and Meijer DK (1998a) Contribution of the murine mdr1a P-glycoprotein to hepatobiliary and intestinal elimination of cationic drugs as measured in mice with an mdr1a gene disruption. *Hepatology* **27:**1056–1063.

Smit JW, Weert B, Schinkel AH, and Meijer DK (1998b) Heterologous expression of various P-glycoproteins in polarized epithelial cells induces directional transport of small (type 1) and bulk (type 2) cationic drugs. *J Pharmacol Exp Ther* **286:**321–327.

Sonnichsen DS, Liu Q, Schuetz EG, Schuetz JD, Pappo A, and Relling MV (1995) Variability in human cytochrome P450 paclitaxel metabolism. *J Pharmacol Exp Ther* **275:**566–575.

Soria I and Zimmerman CL (1994) Intestinal absorption of (−)carbovir in the rat. *Pharm Res (NY)* **11:**261–271.

Stephens RH, O'Neill CA, Warhurst A, Carlson GL, Rowland M, and Warhurst G (2001) Kinetic profiling of P-glycoprotein-mediated drug efflux in rat and human intestinal epithelia. *J Pharmacol Exp Ther* **296:**584–591.

Suttle AB and Brouwer KLR (1995) Regional gastrointestinal absorption of ranitidine in the rat. *Pharm Res (NY)* **12:**1311–1315.

Suttle AB, Pollack GM, and Brouwer KL (1992) Use of a pharmacokinetic model incorporating discontinuous gastrointestinal absorption to examine the occurrence of double peaks in oral concentration-time profiles. *Pharm Res (NY)* **9:**350–356.

Suzuki A, Higuchi WI, and Ho NF (1970a) Theoretical model studies of drug absorption and transport in the gastrointestinal tract. I. *J Pharm Sci* **59:**644–651.

Suzuki A, Higuchi WI, and Ho NF (1970b) Theoretical model studies of drug absorption and transport in the gastrointestinal tract. II. *J Pharm Sci* **59:**651–659.

Suzuki H and Sugiyama Y (2000) Role of metabolic enzymes and efflux transporters in the absorption of drugs from the small intestine. *Eur J Pharm Sci* **12:**3–12.

Svanvik J (1973) Mucosal blood circulation and its influence on passive absorption of the small intestine. *Acta Physiol Scand Suppl* **385:**1–44.

Takamatsu N, Welage SS, Hayashi Y, Yamamoto RY, Barnett JL, Shah VP, Lesko LJ, Ramachandran C, and Amidon GL (2002) Variability in cimetidine absorption and plasma double peaks following oral administration in the fasted state in humans: correlation with antral motility. *Eur J Pharm Biopharm* **53:**37–47.

Tam D, Sun H, and Pang KS (2003a) Influence of P-glycoprotein, transfer clearances, and drug binding on intestinal metabolism in Caco-2 cell monolayers or membrane preparations: a theoretical analysis. *Drug Metab Dispos* **31:**1214–1226.

Tam D, Tirona RG, and Pang KS (2003b) Segmental intestinal transporters and metabolic enzymes on intestinal drug absorption. *Drug Metab Dispos* **31:**373–383.

Tamai I, Saheki A, Saitoh R, Sai Y, Yamada I, and Tsuji A (1997) Nonlinear intestinal absorption of 5-hydroxytryptamine receptor antagonist caused by absorptive and secretory transporters. *J Pharmacol Exp Ther* **283:**108–115.

Tamai I, Sai Y, Ono A, Kido Y, Yabuuchi H, Takanaga H, Satoh E, Ogihara T, Amano O, Izeki S, and Tsuji A (1999) Immunohistochemical and functional characterization of pH-dependent intestinal absorption of weak organic acids by the monocarboxylic acid transporter MCT1. *J Pharm Pharmacol* **51:**1113–1121.

Tanaka K, Hirai M, Tanigawara Y, Yasuhara M, Hori R, Ueda K, and Inu K-I (1996) Effect of cyclosporin analogues and FK506 on transcellular transport of daunorubicin and vinblastine via P-glycoprotein. *Pharm Res (NY)* **13:**1073–1077.

Terao T, Hisanaga E, Sai Y, Tamai I, and Tsuji A (1996) Active secretion of drugs from the small intestine epithelium in rats by P-glycoprotein functioning as an absorption barrier. *J Pharm Pharmacol* **48:**1083–1089.

Terry SI, Gould JC, McManus JP, and Prescott LF (1982) Absorption of penicillin and paracetamol after small intestinal bypass surgery. *Eur J Clin Pharmacol* **23:**245–248.

Thiebault F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, and Willingham MC (1987) Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proc Natl Acad Sci USA* **84:**7735–7738.

Thummel KE, Brimer C, Yasuda K, Thottassery J, Senn T, Lin Y, Ishizuka H, Kharasch E, Schuetz J, and Schuetz E (2001) Transcriptional control of intestinal cytochrome P-4503A by 1-alpha, 25-dihydroxy vitamin D3. *Mol Pharmacol* **60:**1399–1406.

Thummel KW, O'Shae D, Paine MF, Shen DD, Kunze KL, Perkins JD, and Wilkinson GR (1996) Oral first pass elimination of midazolam involves both gastrointestinal and hepatic CYP3A-mediated metabolism. *Clin Pharmacol Ther* **59:**491–502.

Traber PG, Gumucio DL, and Wang W (1991) Isolation of intestinal epithelial cells for the study of differential gene expression along the crypt-villus axis. *Am J Physiol* **260:**G895–G903.

Tsuji A and Tamai I (1989) Na+ and pH dependent transport of foscarnet via the phosphate carrier system across intestinal brush border vesicles. *Biochem Pharmacol* **38:**1019–1022.

Tsuji A and Tamai I (1996) Carrier-mediated intestinal transport of drugs. *Pharm Res (NY)* **13:**963–977.

Ungell A-L, Nylander S, Bergstrand S, Sjoberg A, and Lennernäs H (1998) Membrane transport of drugs in different regions of the intestinal tract of the rat. *J Pharm Sci* **87:**360–366.

Vidon N, Evard D, Godbillon J, Rongier M, Duval M, Schoeller JP, Bernier JJ, and Hirz J (1985) Investigation of drug absorption from the gastrointestinal tract of man. II. Metoprolol in the jejunum and ileum. *Br J Clin Pharmacol* **19 (Suppl 2):**1078–1128.

Wacher VJ, Salphati L, and Benet LZ (2001) Active secretion and enterocytic drug metabolism barriers to drug absorption. *Adv Drug Delivery Rev* **46:**89–102.

Wacher VJ, Silverman JA, Zhang Y, and Benet LZ (1998) Role of P-glycoprotein and cytochrome P450 3A in limiting oral absorption of peptides and peptidomimetics. *J Pharm Sci* **87:**1322–1330.

Walters HC, Craddock AL, Fusegawa H, Willingham MC, and Dawson PA (2000) Expression, transport properties and chromosomal location of organic anion transporter subtype 3. *Am J Physiol* **279:**G1188–G1200.

Wang Y, Roy A, Sun L, and Lau CE (1999) A double-peak phenomenon in the pharmacokinetics of alprazolam after oral administration. *Drug Metab Dispos* **27:**855–889.

Watkins PB, Murray SA, Winkelman LG, Heuman DM, Wrighton SA, and Guzelian PS (1989) Erythromycin breath test as an assay of glucocorticoid-inducible liver cytochrome P-450. *J Clin Investig* **83:**688–697.

Watkins PB, Wrighton SA, Schuetz EG, Molowa DT, and Guzelian PS (1987) Identification of glucocorticoid-inducible cytochrome P-450 in the intestinal mucosa of rats and man. *J Clin Investig* **80:**1029–1036.

Weinberg SL, Burckhardt G, and Wilson FA (1986) Taurocholate transport by rat intestinal basolateral membrane vesicles. Evidence for the presence of an anion exchange transport system. *J Clin Investig* **78:**44–50.

Welling PG (1984) Effects of gastrointestinal disease on drug absorption, in *Pharmadynamic Basis for Drug Treatment* (Benet LZ, Massoud N, and Gambertoglio JG eds) pp 29–47, Raven Press, New York.

Wen Y, Remmel RP, and Zimmerman CL (1999) First-pass disposition of (−)-6-aminocarbovir in rats. I. Prodrug activation may be limited by access to enzyme. *Drug Metab Dispos* **27:**113–121.

Wilson FA and Treanor LL (1975) Characterization of the passive and active transport mechanisms for bile acid uptake into rat isolated intestinal epithelial cells. *Biochim Biophys Acta* **406:**280–293.

Windmueller HG and Spaeth AE (1977) Vascular perfusion of rat small intestine: metabolic studies with isolated and in situ preparations. *Fed Proc* **36:**177–181.

Windmueller HG and Spaeth AE (1981) Vascular autoperfusion of rat small intestine in situ. *Methods Enzymol* **77:**120–129.

Winiwarter S, Bonham NM, Ax F, Hallberg A, Lennernäs H, and Karlén A (1998) Correlation of human jejunal permeability (*in vivo*) of drugs with experimentally and theoretically derived parameters. A multivariate data analysis approach. *J Med Chem* **41:**4939–4949.

Winne D (1977) Shift of pH-absorptin curves. *J Pharmacokinet Biopharm* **5:**53–94.

Witcher JW and Boudinot FD (1996) Applications and simulations of a discontinuous oral absorption pharmacokinetic model. *Pharm Res (NY)* **13:**1720–1724.

Wong MH, Oelkers P, Craddock AL, and Dawson PA (1994) Expression cloning and characterization of the hamster ileal sodium-dependent bile acid transporter. *J Biol Chem* **269:**1340–1347.

Xu X, Hirayama H, and Pang KS (1989) First pass metabolism of salicylamide Studies in the once through vascularly perfused rat intestine-liver preparation *Drug Metab Dispos* **17:**556–563.

Yin OQ, Tomlinson B, Chow AH, and Chow MS (2003) A modified two-portion absorption model to describe double-peak absorption profiles of ranitidine. *Clin Pharmacokinet* **42:**179–192.

Yu LX and Amidon GL (1998) Saturable small intestinal drug absorption in humans: modeling and interpretation of ceftrizine data. *Eur J Pharm Biopharm* **45:**199–203.

Yu LX and Amidon GL (1999) A compartmental absorption and transit time model for estimating oral drug absorption. *Int J Pharm* **186:**119–125.

Yu LX, Lipka E, Crison JR, and Amidon GL (1996) Transport approaches to the biopharmaceutical design of oral drug delivery systems: prediction of intestinal absorption. *Adv Drug Delivery Rev* **19:**359–376.

Zamber CP, Lamba JK, Yasuda K, Farnum J, Thummel K, Schuetz JD, and Schuetz EG (2003) Natural allelic variants of breast cancer resistance protein (BCRP) and their relationship to BCRP expression in human intestine. *Pharmacogenetics* **13:**19–28.

Zhang L, Brett CM, and Giacomini KM (1998) Role of organic cation transporters in drug absorption and elimination. *Annu Rev Pharmacol Toxicol* **38:**431–460.

Zhao YH, Abraham MH, Le J, Hersey A, Luscombe CN, Beck G, Sherborne B, and Cooper I (2002) Rate-limited steps of human oral absorption and QSAR studies. *Pharm Res (NY)* **19:**1446–1457.

Zhao YH, Le J, Abraham M, Hersey A, Eddershaw PJ, Luscombe CN, Boutina D, Beck G, Sherborne B, Cooper I, and Platts JA (2001) Evaluation of human intestinal absorption data and subsequent derivation of a quantitative structure-activity relationship (QSAR) with the Abraham descriptors. *J Pharm Sci* **90:**749–784.

Dr. K. Sandy Pang received the B.Sc. degree from the University of Toronto and Ph.D. degree from University of California at San Francisco and was a Fogarty fellow at the National Institutes of Health. She is presently Professor of Pharmacy and Pharmacology at the Faculties of Pharmacy and Medicine at the University of Toronto. Dr. Pang is known for her work in the fields of pharmacokinetics, drug metabolism, and liver physiology. Her research is aimed toward a mechanistic-based understanding of the handling of drugs and their metabolites within eliminating organs, namely the liver, intestine, and kidney. The approach is via development and formulation of physiologically relevant processes of organ clearances through theoretical treatises and the validation by experimental approaches in vitro (expression systems, cells, and subcellular preparations) and in perfused organs. Her work on metabolic kinetics emphasizes the need for consideration of heterogeneity and the order of sequential processing and has led to the finding that differences exist in the kinetics of formed versus preformed metabolites. Her more recent focus is on extension of the principles learned on hepatic clearances to examine heterogeneity in intestinal absorption and clearance with respect to the first-pass effect. Her recent interests include drug discovery programs in examination of hemoglobin conjugates and effective new drugs in a metastatic rat liver tumor model. She was the recipient of the National Institutes of Health Research Career Development Award, Faculty Development Award from the Medical Research Council of Canada, the McNeil Award from the Faculties of Pharmacies in Canada, and the Research Achievement Award in Pharmacokinetics, Pharmacodynamics, and Drug Metabolism from the American Association of Pharmaceutical Scientists (AAPS).