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Review

# Formed and preformed metabolites: facts and comparisons

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## Abstract

The administration of metabolites arising from new drug entities is often employed in drug discovery to investigate their associated toxicity. It is expected that administration of metabolites can predict the exposure of metabolites originating from the administration of precursor drug. Whether exact and meaningful information can be obtained from this has been a topic of debate. This communication summarizes observations and theoretical relationships based on physiological modelling for the liver, kidney and intestine, three major eliminating organs/tissues. Theoretical solutions based on physiological modelling of organs were solved, and the results suggest that deviations are expected. Here, examples of metabolite kinetics observed mostly in perfused organs that did not match predictions are provided. For the liver, discrepancies in fate between formed and preformed metabolites may be explained by the heterogeneity of enzymes, the presence of membrane barriers and whether transporters are involved. For the kidney, differences have been attributed to glomerular filtration of the preformed but not the formed metabolite. For the intestine, the complexity of segregated flows to the enterocyte and serosal layers and differences in metabolism due to the route of administration are addressed. Administration of the metabolite may or may not directly reflect the toxicity associated with drug use. However, kinetic data on the preformed metabolite will be extremely useful to develop a sound model for modelling and simulations; in-vitro evidence on metabolite handling at the target organ is also paramount. Subsequent modelling and simulation of metabolite data arising from a combined model based on both drug and preformed metabolite data are needed to improve predictions on the behaviours of formed metabolites.

## Introduction

Active or reactive metabolites have been a topic of interest in drug development and post-market surveillance, because of untoward reactions and toxicity associated with bioactivation. The desire to monitor biotransformation products of new drug entities that are biologically relevant metabolite species has become attainable, since powerful instrumentation such as tandem mass spectrometry and accelerator mass spectrometry, which accelerates ions to extraordinarily high kinetic energies before mass analysis to sensitively and accurately analyze elemental and isotopic compositions, renders the quantitation of extremely low levels of metabolites a reality. Hence, there is the urgent need to strategize and validate concepts regarding formed metabolites with regard to risk assessment and safety issues (Davis-Bruno & Atrakchi 2006; FDA 2008).

A classification of metabolites was developed by the late Dr James R. Gillette (Gillette 1980). Stable metabolites, active or inactive, are those that will leave and re-enter the circulation from formation organs, and these are stable enough to be readily quantified. Short-lived metabolites are those that are unstable, but are stable enough to leave the organ of formation to reach other organs. Ultra short-lived metabolites are unstable and exist for a short time within the formation organ. These reactive metabolites are extremely unstable and reactive, and are not detected readily. The reactivity of toxic metabolites is often demonstrated as electrophilic attack on nucleophiles such as glutathione, protein or DNA to form adducts via covalent binding. Another classification is the unique metabolites that are formed only in humans and differ from those observed in animals. Hence, their risk assessment cannot be addressed by testing in the animal species that are ordinarily used for safety evaluation.

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**Table 1** Examples of active and/or toxic metabolites

| Drug | Active/toxic metabolite | References |
|---|---|---|
| Acetaminophen | NAPQI | Dahlin et al 1984 |
| Acetylsalicylic acid | Salicylic acid | Herman 2003 |
| Acetylmethadol | NorLAAM, DiNorLAAM | Stickney 1978; Herman 2003 |
| Allopurinol | Oxipurinol | Drayer 1976 |
| Amitriptyline | Nortriptyline | Nybäck et al 1975 |
| Imipramine | Desipramine | Nybäck et al 1975 |
| Buspirone | 6-Hydroxybuspirone | Wong et al 2007 |
| Capecitabine | 5′-Deoxy-5-fluorocytidine and 5-fluorouracil | Tabata et al 2004 |
| Carbamazepine | Carbamazepine 10-11 epoxide | Bu et al 2005 |
| 2-Hydroxycarbamazepine | 2-Hydroxyiminostilbene and carbamazepine iminoquinone | Pearce et al 2005 |
| Cefotaxime | Desacetyl metabolite | Jones 1995 |
| Choral hydrate | Tricholoroethanol, choral hydrate | Lash et al 2000 |
| Codeine | Morphine | Wilcox & Owen 2000 |
| Cortisone | Cortisol | Edwards & Steward 1991 |
| Diazepzam, prazepam | Nordiazpem, temazepam, oxazepam | Breimer et al 1980 |
| Diclofenac | Benzoquinine intermediate/acylglucuronide | Banks et al 1995; Tan 2003; Poon et al 2001; Grillo et al 2003 |
| 1,1-Dichloroethylene | Epoxide | Simmonds et al 2004 |
| Diflunisal | Acylglucuronide | Wang & Dickinson 1998 |
| Doxorubicin | Doxorubicinol | Olson et al 1988 |
| Glutethimide | 4-Hydroxyglutethimide | Aboul-Enein et al 1975 |
| Ipomeanol | Reactive metabolite | Boyd et al 1978; Alvarez-Diez & Zheng 2004 |
| Isonizid and iproniazid | Acetylhydrazine | Nelson et al 1976 |
| Lidocaine | MEGX, GX | Benowitz & Meister 1978 |
| Lovastatin lactone | Lovastatin acid | Vickers et al 1990 |
| Maxipost | *Ortho*quinone methide reactive metabolite | Zhang 2003 |
| Minoxidil | Minoxidil sulfate | Uno et al 1987; Falany & Kerl 1990 |
| Morphine | Morphine 6-glucuronide | Dahan et al 2008 |
| Nefazodone | Epoxide | Kalgutkar et al 2005 |
| Nelfinavir | Nelfinavir hydroxy-*t*-butylamide | Kirani et al 2004 |
| Norgestimate | 17-Deacetyl norgestimate | McGuire et al 1990 |
| Procainamide | N-Acetylprocainamide | Connolly & Kates 1982 |
| Prednisolone | Prednisone | Arancibia et al 2005 |
| Rofecoxib | Reactive species | Oitate et al 2007 |
| Sulfasalazine | 5-Amino-salicylic acid | Williams & Hallett 1989 |
| Tamoxifen | 4-Hydroxytamoxifen; endoxifen; 4-hydroxy-*N*-desmethyl-tamoxifen, quinone methide | Jordan et al 1977; Lim et al 2005; Fan & Bolton 2001 |
| Telmisartin | Acylglucuronide | Ebner et al 1999 |
| Tolmetin | Acyl-coenzyme A thioester intermediate epoxide | Chen 2006; Olsen et al 2007 |
| Troglitazone | Troglitazone sulfate, reactive intermediate, sulfonium ion | Funk et al 2001; He et al 2004 |
| Valproic acid | 4-Ene valproate | Gopaul et al 2000 |
| Zidovudine | Tri-phosphate metabolite | Barry et al 1996 |
| Zomepirac | Acylglucuronide | Grillo & Hua 2003 |

Numerous metabolites are known to be pharmacologically active and/or toxic (Drayer 1976) (Table 1); hence their monitoring is vital. At what point should metabolites be worrisome? This exists when metabolites are culprits of toxicity or contributors to biological activity.

The synthesis and administration of preformed metabolites to animals and humans for the assessment of toxicity and pharmacological activity has been suggested. Recently, the Guidance from the US Food and Drug Administration (FDA 2008) classified metabolites as major metabolites when the percentage in the circulation exceeds 10%, although previously, the Metabolite in Safety Testing (MIST) document had suggested 25% of drug exposure (Baillie et al 2002). The % exposure, if based on comparisons of area under curve (AUC), is under the assumption that the volumes of distribution of formed metabolite and parent compound are similar. Other reports suggest estimation of the absolute amounts of the metabolites (% dose) (Smith & Obach 2005, 2006). It was further suggested that structural considerations – minor vs major differences in chemical structure – must be considered (Smith & Obach 2005, 2006). The proposition infers similarity in permeability/distribution between the parent drug and metabolite when there are only minor differences in their structures. Transporters, which may be important determinants of the disposition of the parent drug and metabolite, are often omitted in the consideration.

A judicious determination of risk assessment warrants a thorough investigation of the toxic species. The purpose of this review is to present known background information for the comparison between formed and preformed metabolites

that may not share the same kinetics and sometimes even yield different secondary metabolites. Our research group has explored theoretical relationships based on physiological modelling approaches for the liver, kidney and intestine, three important eliminating organs. We have also compared the fates of the formed vs preformed metabolites experimentally within each of the organs/tissue in perfusion studies. Examples of metabolite kinetics that do not match those of the preformed metabolite are presented. In the liver, discrepancies have been explained by the heterogeneity of enzymes, membrane barriers and transporter-mediated uptake. In the kidney, differences have been attributed to the glomerular filtration of the preformed but not the formed metabolite. In the intestine, the complexity of segregated flows to the enterocyte and serosal layers and differences in the extents of intestinal metabolism with respect to the oral versus i.v. routes of administration are addressed. This review should provide mechanistic information on expected differences in the disposition of preformed and generated metabolites that enable judicious decisions to be made on whether the administration of metabolites might be meaningful in toxicity outcomes.

## Sequential elimination within formation organs

The simplest case of metabolism of the precursor drug (P) in the formation of the primary metabolites (M1, M2 and M3) with respective formation rate constants of $k_{m1}$, $k_{m2}$ and $k_{m3}$ is depicted; these metabolites are not further metabolized, but are excreted into urine via $k_e\{m1\}$, $k_e\{m2\}$ and $k_e\{m3\}$, respectively (Figure 1). In this situation, the concentrations of the metabolite appearing in the systemic circulation will reflect those that are formed in the organ.

More often than not, the primary metabolite (Mi) formed from P is immediately metabolized (denoted by the rate constant for metabolite, $k_m\{mi\}$) to form the secondary metabolite Mii (Figure 2A); P is also excreted renally with rate constant $k_e$. The secondary metabolite Mii is not

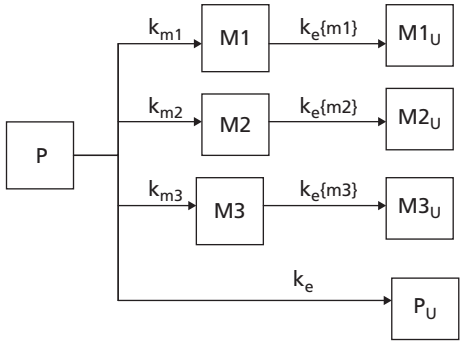


**Figure 1** Schematic depiction of a precursor (P) forming three primary metabolites (M1, M2 and M3) with formation rate constants of $k_{m1}$, $k_{m2}$ and $k_{m3}$, respectively, in a compartmental model. P is excreted unchanged into urine, with rate constant $k_e$. The metabolites are eliminated unchanged only by renal excretion, with excretion rate constants of $k_e\{m1\}$, $k_e\{m2\}$ and $k_e\{m3\}$, respectively. The subscript 'U' denotes the species in urine. (Taken from Pang (1985a) with permission.)

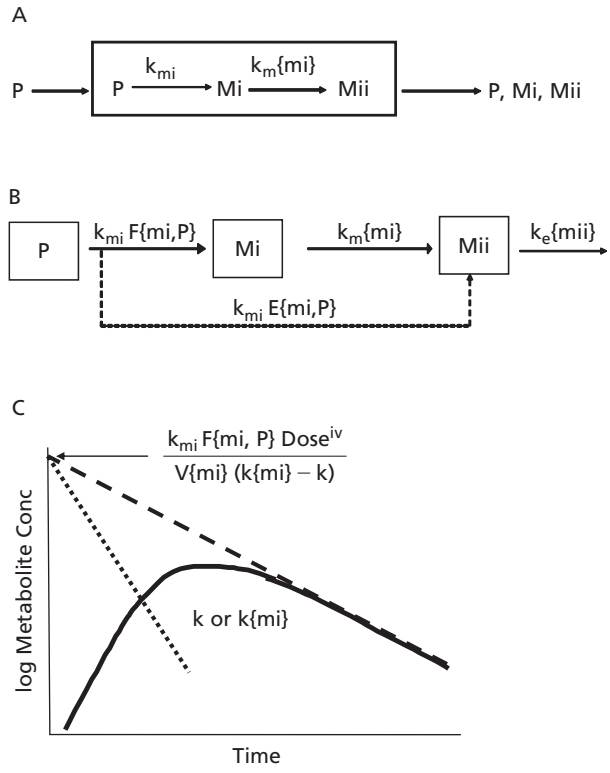


**Figure 2** A. Depiction of sequential metabolism of formed primary metabolite Mi from its precursor, P, within the organ of formation, to the secondary metabolite, Mii, with the formation rate constant $k_m\{mi\}$. B. The equivalent compartmental model showing the respective species, P, Mi and Mii in compartments. The sequential removal of P will give rise to Mii that is detected systemically; the apparent formation rate constant of Mii from the precursor is $k_{mi}E\{mi,P\}$, and the apparent formation rate constant of Mi is $k_{mi}F\{mi,P\}$, where E{mi,P} and F{mi,P} are the extraction ratio and the apparent available fraction of the formed Mi, respectively. C. The intercept on the Y-axis of the semilogarithmic plot of the metabolite concentration vs time profile contains the apparent availability term of the formed metabolite Mi, or F{mi,P}. (Taken from Pang (1985a) with permission.)

metabolized within the formation organ and only undergoes renal excretion; thus, Mii is detected fully in the venous blood. When Mi is only metabolized, its elimination rate constant, k{mi}, equals the metabolic rate constant, $k_m\{mi\}$. For this example, P, Mi and Mii will all appear in the venous blood after the entry of P into the elimination organ. The immediate sequential removal of Mi during its time of genesis within the organ reduces its appearance systemically by the fraction that is removed, the extraction ratio of Mi derived from the precursor or E{mi,P}; this phenomenon is known as 'sequential first-pass elimination of the formed metabolite' (Pang & Gillette 1979). The rate of appearance of the formed metabolite in the circulation is the apparent availability of the formed metabolite (F{mi,P} [or 1 − E{mi,P}]) multiplied by the rate of formation of the metabolite (Pang & Gillette 1979). F{mi,P} is the ratio of the appearance rate of Mi (flow rate [Q] multiplied by the venous concentration of metabolite [$C_{out}\{mi\}$]), whereas the true rate of formation of Mi is Q times the concentration of

metabolite that would have appeared in the venous blood in the absence of sequential elimination ($C\{mi\}_{formed}$).

$$F\{mi,P\} = \frac{QC_{out}\{mi\}}{QC\{mi\}_{formed}} \quad (1)$$

Because the rate of appearance of Mi in the outflow is reduced by E{mi,P}, the effective formation rate constant of the metabolite from the precursor, $k_{fmi}$, is related to the true formation rate constant, $k_{mi}$, as $k_{mi}F\{mi,P\}$ (Pang 1985a). The appearance of Mii in the systemic circulation is partly due to the sequential metabolism of P to Mi then Mii, and partly due to Mi re-entering the organ to form Mii. The concept of sequential first-pass removal of the nascently formed metabolite may be viewed analogously to the first-pass effect in drug absorption, in that a fraction of the dose is removed. The concept conveys that not all formed Mi is detected in the circulation, and that Mii can be formed as a result of P entering the organ to form Mi and then Mii.

How is sequential metabolism of the nascently formed metabolite incorporated into compartmental modelling? First, there should be recognition that the effective formation rate constant of Mi from P is $k_{mi}F\{mi,P\}$ and not $k_{mi}$ (Figure 2B). Second, for mass balance considerations, the sequential removal of P to Mi then Mii reduces the appearance of Mi by the factor E{mi,P}, and directly yields Mii. Thus, there are two sources for Mii formation: one from Mi that is circulating (represented by the metabolic rate constant for Mi, $k_m\{mi\}$, and one from P that is sequentially being metabolized to Mii (represented by $k_{mi}E\{mi,P\}$) (Figure 2B) (St-Pierre et al 1988). For the simplest one-compartment model, the circulating levels of the metabolite may be related to the formation rate constant from precursor as normally considered, except that the F{mi,P} term should also be present (Figure 2C).

The situation becomes more complicated when multiple organs are involved in the formation of Mi. Some organs may form Mi while another organ metabolizes the formed Mi to Mii before these are registered in the systemic circulation. When multiple parallel organs form Mi from P (Figure 3),

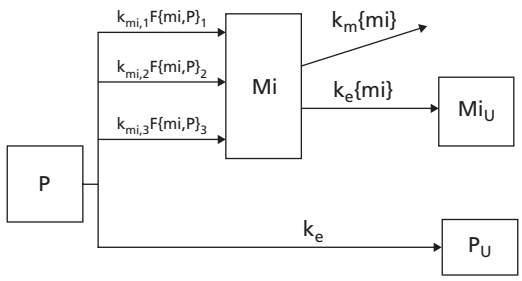


**Figure 3** Depiction of multiple pathways (in three different organs) forming a common primary metabolite, Mi, with the respective formation rate constants of $k_{mi,1}$, $k_{mi,2}$ and $k_{mi,3}$. These rate constants are being modified by sequential elimination (shown as the available fractions, $F\{mi,P\}_1$, $F\{mi,P\}_2$ and $F\{mi,P\}_3$), yielding products that represent the apparent formation rate constants. The Mi formed undergoes both metabolism and excretion, with rate constants $k_m\{mi\}$ and $k_e\{mi\}$, respectively. P is excreted unchanged into the urine with rate constant, $k_e$. The subscript 'U' denotes the species in urine. (Taken from Pang (1985a), with permission.)

the sum of the effective organ formation rate constants gives the overall effective formation rate constant, $k_{fmi}$ (Pang 1985a):

$$k_{fmi} = \sum_{j=1}^{3} k_{fmi,j} = k_{mi,1}F\{mi,P\}_1 + k_{mi,2}F\{mi,P\}_2 + k_{mi,3}F\{mi,P\}_3 \quad (2)$$

and the overall effective apparent availability equals the quotient of the overall apparent formation rate constant, $k_{fmi}$ and the formation rate constant, $k_{mi}$ (Pang 1985a):

$$F\{mi,P\} = \frac{\sum_{j=1}^{n} k_{fmi,j}}{k_{mi}} = \frac{\sum_{j=1}^{n} k_{mi,j}F\{mi,P\}_j}{\sum_{j=1}^{n} k_{mi,j}} \quad (3)$$

## Differences in extraction ratios between formed and preformed metabolites

The compartmental approach that normally renders the least complexity in mathematical presentation is used most frequently in pharmacokinetics. The approach assumes that organs within the body are well stirred, and that there is no gradient of enzyme, transporter or drug/metabolite concentration within the organ. The important question is whether the immediate metabolism/excretion of the formed metabolite, denoted as the extraction ratio (E{mi,P}) or the complementary available fraction (F{mi,P}) are the same as those of the preformed species. In other words, are the extraction ratios of preformed and formed Mi (E{pmi} and E{mi,P}) arising from administration of Mi and P, respectively, equal? The same question may be posed for the secondary metabolite, Mii. For Mii, does E{pmii} equal E{mii,mi} or E{mii,P} arising from the primary metabolite and the precursor, respectively? This is one of the most pertinent questions in metabolite kinetics, since often preformed metabolites are synthesized and administered for investigation of risk assessment and toxicity associated with formed metabolites upon precursor drug bioactivation. However, the theoretical and experimental bases of this strategy are seldom tested. The underlying assumption has been that the preformed metabolite behaves identically to the formed metabolite and reflects the toxicity.

## Differences between the kinetics of generated vs preformed metabolite in the liver

The simplest way to view sequential metabolism is to view the liver as the only eliminating organ, as in the perfused liver preparation (Figure 4). The physiological model is often used to inter-relate the flow, tissue (defined by volume), vascular and tissue binding, and events such as transport and metabolism (de Lannoy & Pang 1993; de Lannoy et al 1993). The intrinsic clearances for metabolism and excretion are denoted by $CL_{int,met,L}$ and $CL_{int,sec,L}$, respectively; those for

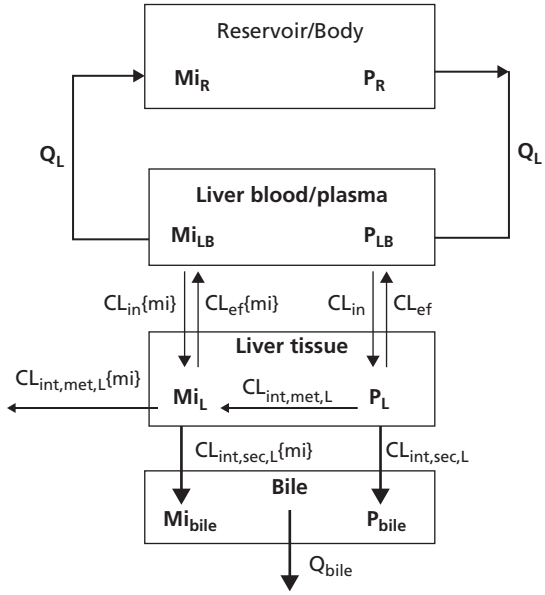


**Figure 4** A physiologically based model representing the liver perfusion system, in which the reservoir (R), liver blood (LB), liver (L) and bile compartments are represented. The influx ($CL_{in}$) and efflux ($CL_{ef}$) clearances at the basolateral membrane denote entry and exit of the precursor drug (P) or metabolite {Mi} between blood and liver tissue. Metabolism and excretion into bile occur with metabolic intrinsic clearance, $CL_{int,met,L}$ and $CL_{int,sec,L}$, respectively. Parameters describing Mi are further qualified by '{mi}'. $Q_{bile}$ denotes the bile flow rate.

influx and efflux at the basolateral membrane are denoted as $CL_{in}$ and $CL_{ef}$, respectively. These parameters for the metabolite are expected to be the same for Mi regardless of whether the metabolite is preformed or generated, suggesting the same transport and metabolic activities for both. The same is assumed to be true for binding of the metabolites, denoted as the unbound fraction in blood ($f_u${mi}). This type of physiologically based model serves to describe sequential metabolism well, since inclusion of the tissue compartment allows for the occurrence of sequential elimination. In this physiologically based model, the equations for the extraction ratio of precursor drug, preformed and formed metabolites have been solved (de Lannoy & Pang 1993; de Lannoy et al 1993). The solutions derived predict that, in the absence of a barrier, the formed and preformed metabolites would exhibit the same extraction ratio; however, in the presence of a barrier, the extraction of the formed metabolite far exceeds that of the preformed metabolite (Table 2). It is noted further that AUC{pmi} is independent of drug parameters, whereas AUC{mi,P} depends on both the intrinsic clearances of metabolism and secretion of the precursor (Table 2).

### *Differences in metabolite kinetics due to zonal distribution of enzymes in the liver*

Many solutes are lipophilic, and the $CL_{in}$ values as well as $CL_{ef}$ values for the parent drug and metabolite are much greater than the $CL_{int,met}$ and $CL_{int,sec}$ of the liver (Figure 4). For lipophilic substrates and metabolites that exhibit flow-limited distribution (absence of barrier effect), the expectation is that E{mi,P} equals E{pmi}, as shown in Table 2. However, evidence contrary to this view is prevalent in the literature. One main reason is the heterogeneous distribution of enzymes and transporters in the the liver lobule. Enzyme heterogeneity within zonal regions of the liver is one of the most important determinants that dictate the type of primary and secondary metabolites formed and the extent of sequential metabolism. The microcirculatory zones 1, 2, and 3 that are defined with respect to the intralobular oxygen gradient by Rappaport (1958) are synonymous with the metabolic zones, periportal, midzonal and perivenous regions

**Table 2** Equations denoting the area under the curve (AUC) and extraction ratios of the drug precursor (parameters not further qualified), the preformed (pmi) and formed (mi,P) primary metabolite, in the absence and presence of a sinusoidal barrier[a]

| | No barrier | With barrier |
|---|---|---|
| AUC{pmi} | $\frac{Dose\{pmi\}(f_u\{mi\}CL_{int,L}\{mi\}+Q_L)}{Q_L f_u\{mi\}CL_{int,L}\{mi\}}$ | $\frac{Dose\{pmi\}\left[f_u\{mi\}CL_{in}\{mi\}CL_{int,L}\{mi\}+Q_L(CL_{ef}\{mi\}+CL_{int,L}\{mi\})\right]}{Q_L f_u\{mi\}CL_{in}\{mi\}CL_{int,L}\{mi\}}$ |
| AUC{mi,P} | $\frac{Dose\ CL_{int,met,L}}{(CL_{int,met,L}+CL_{int,sec,L})f_u\{mi\}CL_{int,L}\{mi\}}$ | $\frac{Dose\ CL_{int,met,L}CL_{ef}\{mi\}}{(CL_{int,met,L}+CL_{int,sec,L})f_u\{mi\}CL_{in}\{mi\}CL_{int,L}\{mi\}}$ |
| $E\{pmi\}$ | $\frac{f_u\{mi\}CL_{int,L}\{mi\}}{f_u\{mi\}CL_{int,L}\{mi\}+Q_L}$ | $\frac{f_u\{mi\}CL_{int,L}\{mi\}CL_{in}\{mi\}}{f_u\{mi\}CL_{in}\{mi\}CL_{int,L}\{mi\}+Q_L\left(CL_{ef}\{mi\}+CL_{int,L}\{mi\}\right)}$ |
| $E\{mi,P\}$ | = E{pmi} | $\frac{CL_{int,L}\{mi\}[Q_L+f_u\{mi\}CL_{in}\{mi\}]}{Q_L\left(CL_{ef}\{mi\}+CL_{int,L}\{mi\}\right)+f_u\{mi\}CL_{int,L}\{mi\}CL_{in}\{mi\}}$ |
| $\frac{E\{mi,P\}}{E\{pmi\}}$ | 1 | $1+\frac{Q_L}{f_u\{mi\}CL_{in}\{mi\}}$ |

[a] $f_u$ denotes the unbound fraction in blood and $CL_{int,L}$, the total intrinsic clearance (metabolic + excretion) of the liver for the precursor (P) and metabolite, mi, qualified by {mi}; $CL_{in}$ is the influx intrinsic clearance for sinusoidal entry from blood to liver; $CL_{ef}$ is the basolateral efflux intrinsic clearance. These intrinsic clearances for formed and preformed metabolite and the unbound fractions are assumed to be identical. $Q_L$ is the liver blood flow.

**Table 3** Heterogeneity of enzymes observed in animals (mostly rodents) and some human livers. Definitions of enzyme abbreviations are given in the glossary.

| Localization of metabolic activities | | | Examples | References |
|---|---|---|---|---|
| Anterior | Even | Posterior | | |
| | Phenol sulfotransferase SULT1A1 | | Acetaminophen<br>7-Hydroxycoumarin (rodents and human) | Pang & Terrell 1981b; Conway et al 1982; El Mouehlhi & Kauffman 1986 |
| Hydroxysteroid sulfotransferase Sult2a1 | | | | Homma et al 1997; Tan & Pang 2001 |
| | | Estrogen sulfotransferase Sult1e1 | Estrone | Homma et al 1997; Tan & Pang 2001 |
| | | UGT (rodents and human) | Harmol<br>7-Hydroxycoumarin | Pang et al 1983; Conway et al 1984; Knapp et al 1988 |
| | | Cyp P4501a<br>Cyp1e1<br>Cyp3a | Phenacetin | Baron et al 1981,1987; Bengtsson et al 1987; Ignelman-Sundberg et al 1988; Pang et al 1988a; Oinonen & Lindros 1995 |
| | | Cytochrome P450 | | |
| | | Epoxide hydrolase | | Baron et al 1980 |
| | | Carboxylesterase | Enalapril | Pang et al 1991; Abu Zahra & Pang 2000 |
| | | Glutathione S-transferase (rodents and human) | Bromosulfophthalein<br>Ethacrynic acid | Redick et al 1982; Zhao et al 1993<br>Tirona et al 1999 |
| | | Glutathione peroxidase | | Kera et al 1987 |
| | | N-acetyl transferase 2 | | Stanley et al 1997 |
| | | Carboxylesterases | | Pang et al 1991 |
| | Arylsulfatase C | | | Anundi et al 1986; Tan & Pang 2001 |
| | | Glycine N-acetyltransferase | Benzoic acid | Chiba et al 1994 |

respectively, and contain varying amounts of enzymes. Direct and indirect techniques have shown an enriched perivenous abundance of the cytochrome P450s, epoxide hydroxylase, glutathione-S-transfersases and carboxylesterases, and either an even or perivenous abundance of the uridine diphosphate glucuronosyltransferases (UGTs). The distribution of the sulfotransferases, however, is highly isoform dependent (Table 3). A similar comment can be made concerning transporters and their distributions (discussed below). The metabolism and excretion of a substrate or metabolite can occur only when it gains access into hepatocytes from the sinusoid, either passively or via sinusoidal transporters. Given the marked enzyme heterogeneities noted for phase I and II reactions, the nature and proportion of primary or secondary metabolites are expected to differ.

[$^{14}$C]Phenacetin, delivered under tracer concentrations in a single-pass fashion to the perfused rat liver preparation, displays sequential metabolism to [$^{14}$C]acetaminophen and [$^{14}$C]acetaminophen sulfate (Pang & Gillette 1978). Even though acetaminophen readily enters the liver (Pang et al 1995), the steady-state extraction ratio of formed [$^{14}$C]acetaminophen (E{mi,P}) is significantly less than that of preformed [$^{3}$H]acetaminophen (E{pmi}) given simultaneously ($0.42 \pm 0.1$ vs $0.67 \pm 0.07$, mean ± s.d., $P < 0.05$). This is explained by the prevalence of upstream (periportal) sulfotransferase activity for acetaminophen sulfation and the downstream (perivenous) O-deethylation activity mediated by Cyp1a2 for acetaminophen formation. This enzyme pattern reduces exposure of the formed acetaminophen to the sulfotransferases. This hypothesis was validated by retrograde single-pass perfusion (Pang & Terrell 1981a), when both [$^{14}$C]phenacetin and [$^{3}$H]acetaminophen were delivered backwards from the hepatic vein to the portal vein (instead of the normal direction of portal vein to hepatic vein), resulting in a reversal of enzyme distributions to render an upstream Cyp1a2 and the sulfotransferases downstream. Under this condition of retrograde perfuson, the extraction ratios of E{mi,P} and E{pmi) for acetaminophen became similar (Pang & Terrell 1981a).

Other examples revolve around the competition reactions between the low-affinity, high-capacity glucuronidation system and the high-affinity, low-capacity upstream sulfation system. As confirmed by retrograde-perfusion studies, glucuronidation activities are downstream of the sulfation activities for several planar substrates such as harmol (Pang et al 1981, 1983; Koster et al 1982), gentisamide (Morris et al 1998a, b) and salicylamide (Xu & Pang 1989; Xu et al 1990). With increasing concentrations of these substrates, formation of the sulfate conjugate(s) decreased, but the proportions of formed glucuronide increased disproportionately to the incoming concentration. The observations were attributed to the higher $K_m$ and more downstream glucuronidation system. Gentisamide, when administered as such, formed mostly the gentisamide 5- and 2-sulfate conjugates (GAM-2S and GAM-5S), and to a lesser extent, the 5-glucuronide (GAM-5G). The gentisamide generated after administration of the parent drug, salicylamide, was metabolized predominantly to GAM-5G. This astounding observation was due to the close proximity of the cytochrome P450 and the Ugt enzymes, as well as the lower

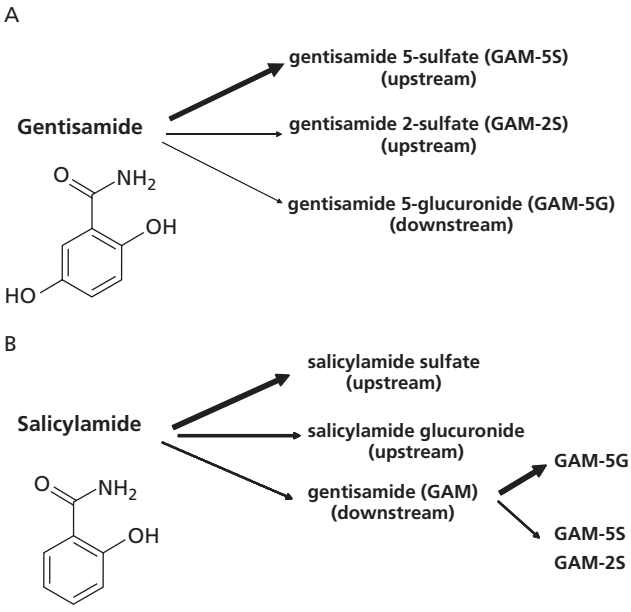


**Figure 5** Schematic depiction of different abundance of enzymes in the periportal (upstream) and perivenous (downstream) zones in the formation of gentisamide metabolites. Because sulfotransferases are upstream of the UDP-glucuronosyltransferases, gentisamide (GAM) formed more of the 5-sulfate (GAM-5S) and 2-sulfate (GAM-2S) metabolites than the 5-glucuronide metabolite, GAM-5G, when administered (A). The situation is reversed when the precursor salicylamide is added to the liver preparation, since GAM is formed downstream by the cytochrome P450s; thus, GAM formed more GAM-5G and much less of the sulfate conjugates, GAM-5S and GAM-2S, before exiting the liver (B).

abundance of sulfotransferases downstream, the site of formation of gentisamide (Morris et al 1988b; Schwab et al 2001) (Figure 5). The different metabolite formation patterns clearly show that the nature of secondary metabolites formed from administration of the primary metabolite or the precursor drug into the liver can differ.

### *Differences in metabolite kinetics due to sequestered space for metabolism*

It must be recognized that substrates in the cytosol need to reach the endoplastic reticulum (ER) to access the cytochrome P450 enzymes for oxidation and the Ugts for glucuronidation, or the mitochondria for amidation (Tirona & Pang 1996). For substrates that do not partition readily between the ER and cytosol, the ER will appear as a 'sequestered space' that promotes the coupling of enzymatic reactions. Hydroxylated products may be more readily glucuronidated sequentially since the enzymes are in close proximity, and sulfation is a lesser occurrence since sulfotransferases are in the cytosol. This was observed for salicylamide hydroxylation to form gentisamide, and the subsequent glucuronidation to GAM-5G, as discussed above (Tirona & Pang 1996).

Another example is provided by estrone ($E_1$) and estrone sulfate ($E_1S$). Upon administration of $E_1S$ as the precursor, desulfation to $E_1$ by sulfatases, followed by the preferential glucuronidation to estrone glucuronide ($E_1G$), occurred in the ER (Figure 6). For $E_1S$ and $E_1$ that undergo futile cycling, parallel decay profiles for $E_1S$ and $E_1$ are expected following their administration into the recirculating perfused rat liver preparation, even after consideration of zonation for estrone sulfation and glucuronidation (perivenous) and estrone desulfation (even distribution) (Tan et al 2001). This parallel decay pattern was observed after administration of $E_1S$ but not $E_1$ (Figure 7A). The observation was explained by the rapid equilibration of $E_1S$ but not $E_1$ between the ER space and cytosol. For $E_1$, efflux from the ER to the cytosol was slower, and this resulted in different half-lives for $E_1S$ and $E_1$. The lack of rapid equilibration of $E_1$ between the ER space and the cytosol deterred the rapid re-sulfation of $E_1$ to form $E_1S$ in the cytosol (Tan et al 2001). Instead, the close proximity of the sulfatase and Ugts in the ER rendered a higher glucuronidation of $E_1$ (Tan et al 2001). When the efflux of $E_1$ from the ER was rendered faster in simulations, the decay profiles following administration simulated for both $E_1S$ and $E_1$ became parallel (Figure 7B).

### *Differences in metabolite kinetics due to sequential metabolism*

An important question that is often asked is whether precursor kinetics affect the behaviour of the formed metabolite, namely, the extent of sequential metabolism of the formed metabolite. First, it is recognized that the mean residence time of a formed metabolite includes that of the precursors (Chan et al 1985; St-Pierre & Pang 1993a, b). Second, the rate of metabolism of the metabolite that is already in existence (preformed) will be the fastest (Pang et al 1985a). When the metabolite is formed rapidly by a fast rate constant, sequential metabolism of the formed metabolite is also rapid. However, when the rate constant for formation of the primary metabolite is slow, the rate of metabolism of the formed metabolite also becomes slower. Hence, the rate constant for formation of the metabolite ($k_m$) can modulate the rate of sequential metabolism: the faster the $k_m$, the greater the extent of sequential metabolism (Figure 8). Increasing the number of steps involved in formation of the metabolite will therefore decrease the extent of sequential metabolism, in as much as the steps delay formation of the metabolite (Pang 1985b). This was observed in the sequential metabolism of diazepam to its primary metabolites, nordiazepam or temazepam, then to secondary metabolite (Mii), oxzaepam, which forms a glucuronide conjugate (Figure 9). The extraction ratio of the preformed secondary metabolite exceeds those coming from Mi or P: E{pmii} > E{mii,mi} and E{mii,P} (St-Pierre & Pang 1993a, b). Another factor that may affect the extent of sequential metabolism is the flow rate. In the liver that forms sulfates upstream and glucuronides downstream, the flow rate to the organ will affect the transit time and therefore the contact time with enzymes. A faster flow rate will result in shortening of the transit time in formation of upstream metabolite, sparing more substrate for downstream metabolism (Dawson et al 1985; Chen & Pang 1997).

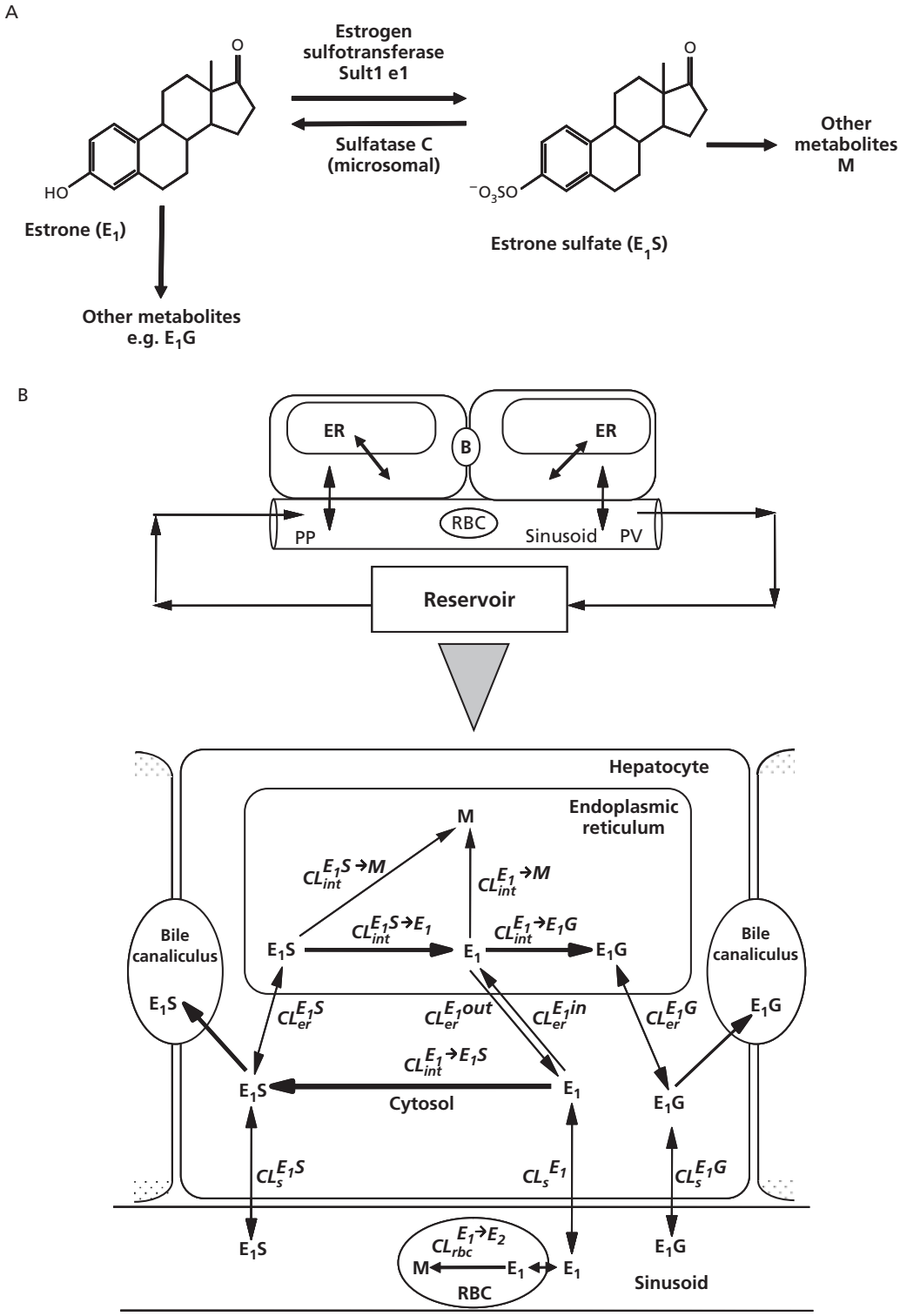


**Figure 6** A. Depiction of futile cycling between estrone ($E_1$) and estrone sulfate ($E_1S$) via estrogen sulfotransferase (Sult1e1) and sulfatase C in the rat liver. B. Modelling of data obtained from Tan et al (2001), with consideration of desulfation (intrinsic clearance, $CL_{\text{int}}^{E_1S\rightarrow E_1}$) and glucuronidation (intrinsic clearance $CL_{\text{int}}^{E_1\rightarrow E_1G}$) taking place in the endoplasmic reticulum (ER). The equilibration of $E_1S$ between cytosol and the ER is rapid, whereas the efflux of $E_1$ out of the ER is slower ($CL_{\text{er}}^{E_1out}$) than that for influx from the cytosol ($CL_{\text{er}}^{E_1in}$); $E_1$ undergoes sulfation (intrinsic clearance $CL_{\text{int}}^{E_1\rightarrow E1S}$) in the cytosol. Binding of $E_1S$ to red blood cell (RBC), and exchange of $E_1S$, $E_1$ and $E_1G$ between sinusoid and cytosol are shown. $E_1S$ is excreted into the bile. (Taken from Tan et al (2001), with permission.)

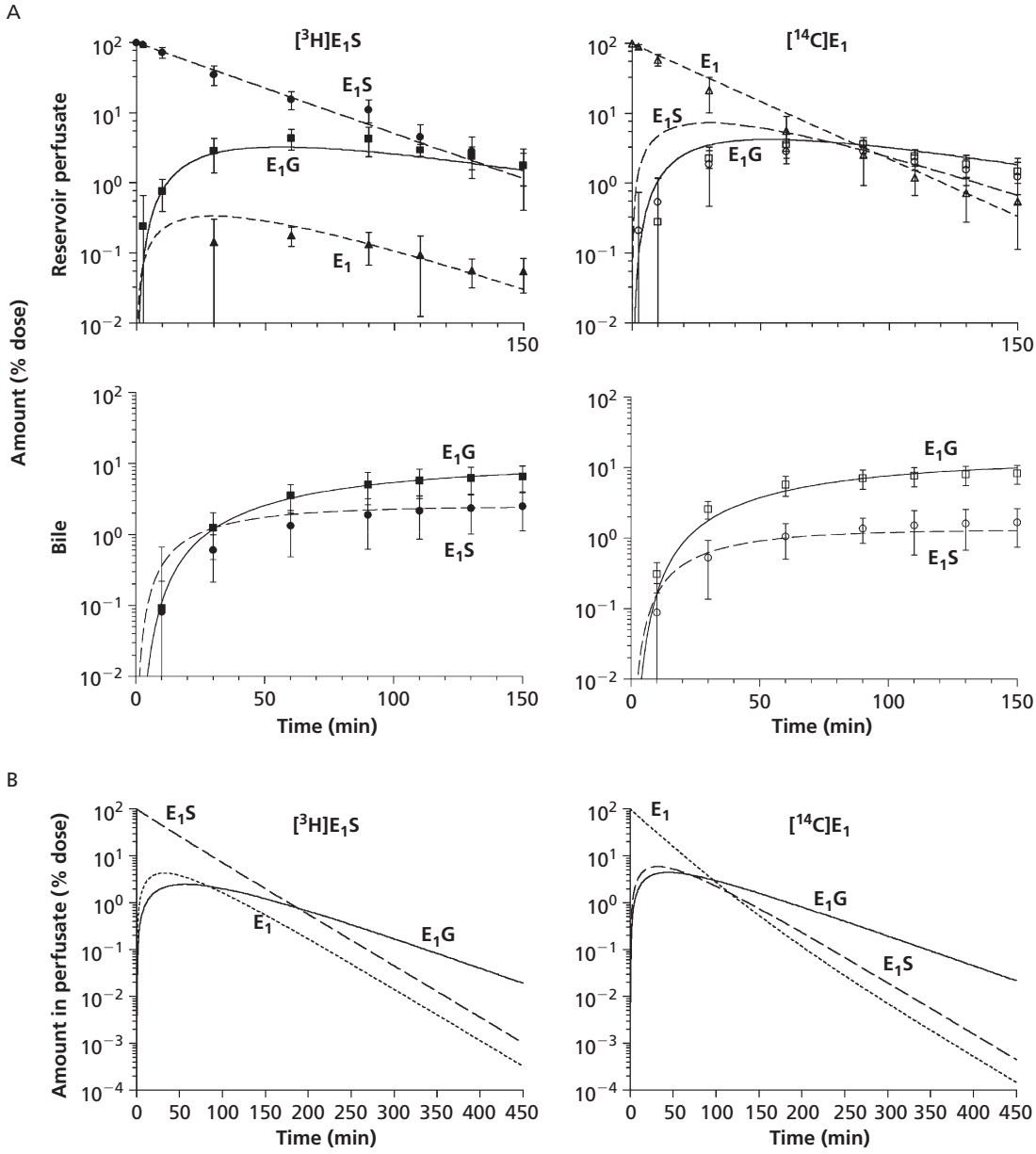


**Figure 7** A. Observed profiles of the amounts of the administered [$^3$H]estrone sulfate ($E_1S$) and the metabolites, estrone glucuronide ([$^3$H]$E_1G$) and estrone ([$^3$H]$E_1$), in perfusate and bile vs time (left) and [$^{14}$C]$E_1$ and its metabolites, [$^{14}$C]$E_1S$ and [$^{14}$C]$E_1G$ (right), in the recirculating perfused rat liver preparations (Tan et al 2001). Note the parallel decay profiles of [$^3$H]$E_1S$ and [$^3$H]$E_1$ when [$^3$H]$E_1S$ was administered to the rat liver but not when [$^{14}$C]$E_1$ was added to the reservoir for liver perfusion. Also note that the extent of [$^{14}$C]$E_1G$ excreted into bile exceeded that of [$^{14}$C]$E_1S$ when [$^{14}$C]$E_1$ was added; the opposite was observed when [$^3$H]$E_1S$ was added. The reason for this is the lack of rapid equilibration of $E_1$ between the endoplasmic reticulum (ER) and the cytosol. B. Decay profiles for $E_1$ and $E_1S$ became parallel when $CL_{er}^{E_1 out}$ was faster to enable rapid equilibration between the ER space and cytosol (taken from Tan et al (2001), with permission).

*Differences in metabolite kinetics due to diffusion barriers and transporters*

It is being increasingly recognized that the sinusoidal membrane plays a strategic role in regulating substrate entry to access enzymes and apical transporters for excretion. Transport barriers for the drug restrict entry and therefore removal; the same applies to the preformed metabolite (de Lannoy & Pang 1986, 1987). Differences between E{mi,P} and E{pmi} are expected when sinusoidal transporters, whose activities are described by the basolateral influx ($CL_{in}$) or efflux ($CL_{ef}$) clearances, exist for drug and metabolite (Figure 4). Low values in relation to the flow rate suggest the presence of a diffusion barrier; values that are higher and saturable suggest that transporters are involved in transport. The equations for E{mi,P} and E{pmi}, with $CL_{in}$ and $CL_{ef}$ representing the transmembrane clearances, have been solved for the liver (de Lannoy et al 1993). As shown in Table 2, differences were found between AUC{mi,P} and AUC{pmi}, and between

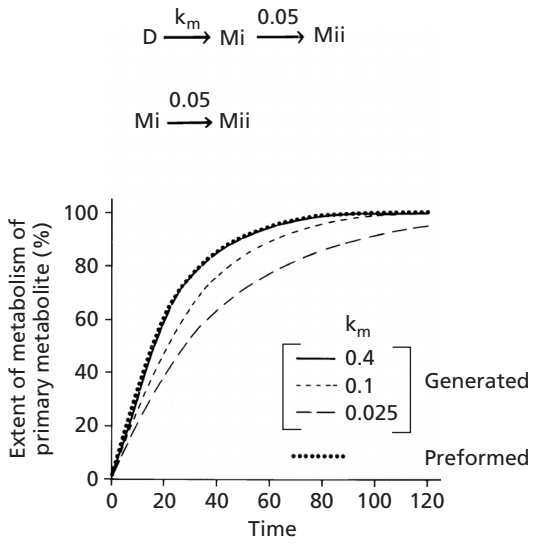


**Figure 8** Effect of precursor formation rate constant, $k_m$, on the extent of sequential metabolism of the primary metabolite that is eliminated at a rate constant of 0.05 time$^{-1}$. Note that in the in vitro system, when the substrate is able to access the enzyme, the preformed metabolite undergoes the most extensive metabolism, followed by the precursor of the highest $k_m$; the extent of sequential metabolism is reduced with lower $k_m$ (modified from Pang (1985a)).

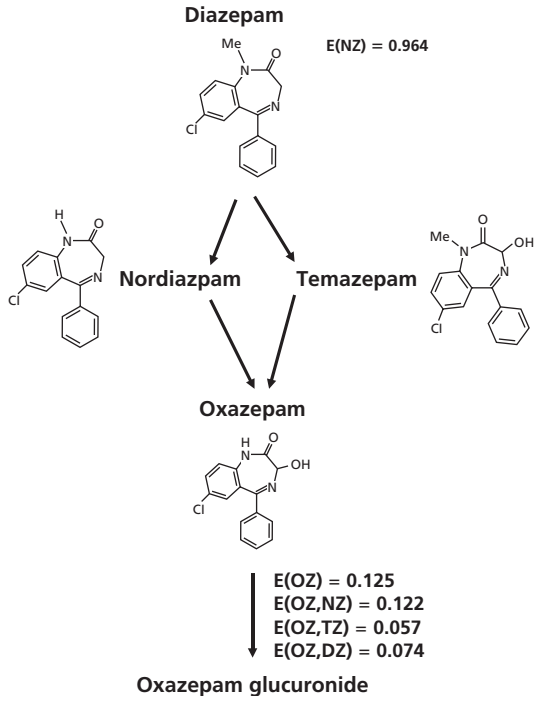


**Figure 9** Depiction of the sequential metabolism of diazepam (DZ) to form nordiazepam (NZ) and temazepam (TZ) by N-demethylation and hydroxylation, respectively. NZ and TZ in turn form oxazepem by hydroxylation and N-demethylation, respectively. The extraction ratio of preformed oxazepam (E{OZ}) to form oxazepam glucuronide is highest (0.125) in the perfused murine liver preparation, whereas these are progressively reduced when NZ, TZ or DZ are used as precursors.

E{mi,P} and E{pmi} derived from the physiological model. The AUC{pmi} depends only on metabolite parameters, whereas AUC{mi,P} also depends on drug parameters (Table 2). When the distribution of the metabolite is flow-limited, that is, $CL_{in}\{mi\}$ is significantly higher than the liver blood flow ($Q_L$), E{mi,P} and E{pmi} will be identical. However, when $CL_{in}\{mi\}$ is small in relation to $Q_L$, the presence of a barrier for the metabolite is inferred, and E{mi,P} will greatly exceed E{pmi} (Table 2).

*Barriers*

Most phase II metabolites are of higher polarity than their parent compounds and are readily excreted into bile (Table 4). In the absence of transporter-mediated sinusoidal entry, the high polarity of many of these phase II metabolites, exemplified by morphine glucuronide and the glutathione conjugates of ethacrynic acid and bromoisovalerylurea, experience poor permeation across the sinusoidal membrane, with E{mi,P} >> E{pmi} as the expected outcome. For the same reason, choleresis is associated with the 4-methylumbelliferyl glucuronide (4MUG) formed when the precursors, 4-methylumbelliferone and 4-methylumbelliferyl sulfate (4MUS), were administered into the perfused liver preparation, but not when preformed 4MUG, a polar compound, was administered (Ratna et al 1993). The same applies to acetaminophen sulfate and salicylamide sulfate conjugates: E{mi,P} >> E{pmi} was again observed. However, for 4MUS, which enters the liver via a transporter, likely the organic anion transporter 2 (OAT2), the extraction ratio and the extent of excretion of preformed 4MUS were similar to values when the lipophilic precursor, 4MU, was administered (Table 3). Another well-studied example is enalaprilat, the active metabolite of the angiotension-converting enzyme inhibitor enalapril, formed via ester hydrolysis. The extraction ratio of formed enalaprilat (0.33) greatly exceeded that from preformed enalaprilat (0.05) in single-pass rat liver perfusion studies (de Lannoy et al 1993) (Figure 10; Table 4). It may certainly be inferred that, because of the different extents of entry, tissue distributions of formed and preformed enalaprilat differed, and the extent of excretion of enalaprilat also differed. The preformed metabolite is unable to enter tissue sites to become distributed, metabolized or excreted or to exert its pharmacologic effect. However, similar conclusions may not be made readily for another similar precursor–product pair, temocapril and temocaprilat, substrates of of the sinusoidal transporter, Oatp1a1, in the rat liver (Ishizuka et al 1998).

*Transporters*

Transporters will further contribute to observed differences in the kinetics of formed and preformed metabolite (Figure 11). Transporters that mediate influx as well as efflux at the basolateral membrane and excretion/secretion at the apical or canalicular membrane strongly influence drug and metabolite removal (Liu & Pang 2006). The sinusoidal transporters include the sodium-dependent taurocholate cotransporting polypeptide (NTCP), which was originally thought of as a dedicated transporter of only bile acids (Hagenbuch & Meier 1994) but has now been found to transport several statins as well (Fujino et al 2005; Ho et al 2006). The organic anion transporting polypeptide (OATP) transports a host of

**Table 4** Effect of poor transmembrane permeability (presence of a membrane barrier) on the extents of elimination of preformed vs formed metabolites in rat liver perfusion studies

| | Extraction ratio of preformed metabolite | Extraction ratio of formed metabolite | | References |
|---|---|---|---|---|
| | E{pmi} | E{mi,P} | Precursor | |
| Enalaprilat | 0.02 | 0.33 | Enalapril | de Lannoy et al 1993 |
| Morphine 3$\beta$-glucuronide | 0.037 | 0.55–0.98 | Morphine | Doherty et al 2006 |
| 4-Methylumbelliferyl glucuronide (150–460 $\mu$M) | 0.073 | 0.66–0.31<br>0.83–0.47 | 4-Methylumbelliferone (64–380 $\mu$M)<br>4-Methylumbelliferone sulfate (99–850 $\mu$M) | Ratna et al 1993 |
| 4-Methylumbelliferyl sulfate (150–460 $\mu$M) | 0.014 | 0.055<br>0.015 | 4-Methylumbelliferone (64–380 $\mu$M)<br>4-Methylumbelliferone glucuronide (150–460 $\mu$M) | Ratna et al 1993 |
| Salicylamide sulfate | 0.0022 | 0.01 | Salicylamide (trace) | Xu & Pang 1989; Xu et al 1990, 1994 |
| Acetaminophen sulfate | 0.0074 | 0.032 | Acetaminophen (0.2–0.6 $\mu$M) | Pang & Terrell 1981; Goresky et al 1992 |
| Bromoisovalerylurea glutathione conjugate: | | | | |
| R-IUSG | 0.0003 | 0.79 | (S)-Bromoisovalerylurea | Polhuijs et al 1991 |
| S-IUSG | 0.0003 | 1.00 | (R)-Bromoisovalerylurea | Polhuijs et al 1993 |
| Ethacrynic acid glutathione conjugate | poor | 0.88 | Ethacrynic acid | Tirona et al 1999 |

substrates (Jacqemin et al 1994), including large anionic compounds, some neutral compounds and even basic compounds (van Montfoort et al 2003; Smith et al 2005). Other transporters include OAT2 and organic cation transporter 1 (OCT1). For biliary excretion, ATP-binding cassette proteins such as the multidrug resistance protein 1 (MDR1), also known as the P-glycoprotien (P-gp), the multidrug resistance-associated protein 2 (MRP2), the breast cancer resistance protein (BCRP) and the bile salt export pump (BSEP), remove the associated substrates into bile. Lateral membrane transport is mediated by MRP3 and MRP4 (Mizuno et al 2003). Vectorial transport and enhancement in removal occurs due to facilitation in entry as well as excretion. Moreover, transport heterogeneity could greatly affect drug clearance (Liu & Pang 2006).

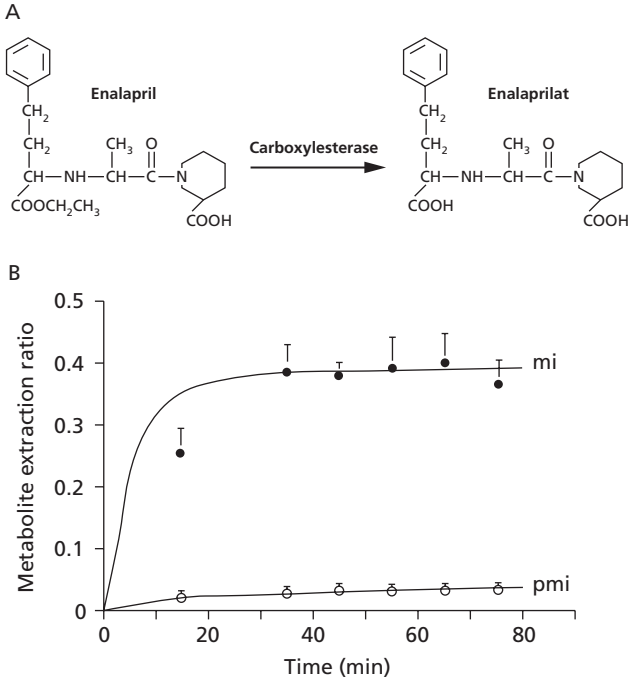


**Figure 10** A. Depiction of the simple metabolic scheme of enalapril undergoing ester hydrolysis by carboxylesterases to its metabolite, enalaprilat. B. The observed higher extraction ratio of the formed metabolite (mi; •) vs that of the preformed metabolite (pmi; ○) in the single-pass perfused rat liver preparation was attributed to the presence of a barrier for enalaprilat. (Taken from de Lannoy & Pang (1993), with permission.)

The glutathione conjugate of bromosulfophthalein (BSPGSH) is a substrate of rat Oatp1a1 (Pang et al 1998a) and is excreted by Mrp2 (Geng et al 1998). When added to the perfused rat liver, the extraction ratio was found to vary from 0.8 to 0.2 with increasing BSPGSH concentration due to saturation of transport processes (Geng et al 1995). When the precursor bromosulfophthalein (BSP), a high-affinity Oatp1a1 substrate (Pang et al 1998a), was added to the single-pass perfused rat liver preparation at low concentrations, entry of BSP was rapid, but BSPGSH was absent from venous blood, suggesting a lack of role for Mrp3 or Mrp4 for basolateral efflux, rendering the apparent extraction ratio of BSPGSH (E{mi,P}) to be unity (Snel et al 1993; Zhao et al 1993). This example shows differences between E{mi,P} and E{pmi} due to the lack or presence of transporters for basolateral efflux and excretion of the metabolite.

## Differences in extraction ratios between generated vs preformed metabolite in the kidney

The basolateral and luminal (brush border) influx and efflux transporters are shown in Figure 12. Major basolateral influx transporters are made up of the OATs and OCTs, whereas MRP2, MRP4, P-gp and BCRP are luminal efflux transporters (Mizuno et al 2003; van Montfoort et al 2003; van de Water et al 2005); the oligopeptide transporters, PEPT1 and PEPT2, are reabsorptive transporters. Less has been

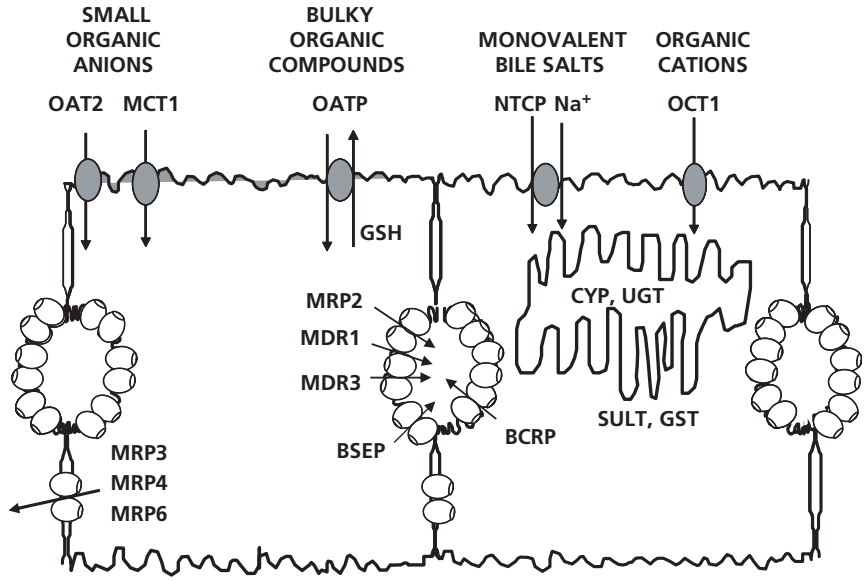


**Figure 11** Schematic depiction of sinusoidal uptake transporters: OAT2, MCT1, OATPs, NTCP and OCT1; efflux transporters: MRP3, MRP4, MRP6; and canalicular transporters: MRP2, MDR1, MDR3, BSEP and BCRP for the human liver. (Modified from Liu & Pang 2006). Definitions of transporter abbreviations are given in the glossary. GSH, glutathione.

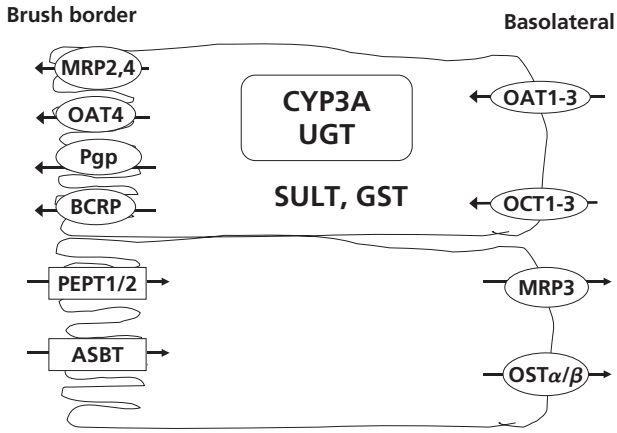


**Figure 12** Renal transporters at the apical and basolateral membranes, respectively. See text for details. Definitions of transporter abbreviations are given in the glossary. P-gp, P-glycoprotein.

published to compare the kinetics of formed and preformed metabolites in the kidney, an organ endowed with metabolic enzymes capable of biotransformation. Again, the simplest way to view renal metabolism/excretion is with the isolated perfused kidney preparation. The physiological model consists of the renal plasma flow, which arises from the reservoir or body and perfuses the renal tissue (Figure 13). Cellular metabolism is denoted by $CL_{int,met,K}$, and excretion and reabsorption at the luminal membrane by $CL^{l}_{ef}$ and $CL^{l}_{in}$, respectively; the influx and efflux clearances at the basolateral membrane are denoted as $CL^{b}_{in}$ and $CL^{b}_{ef}$, respectively. The metabolite may be subject to renal metabolism and renal excretion and reabsorption. The intrinsic clearances of the metabolite are expected to be the same for the preformed and generated metabolite species; the same comment is assumed to be true for binding, denoted as the unbound fraction in blood ($f_u\{mi\}$).

The renal model differs from the liver model (Figure 4) since the kidney filters the drug and preformed metabolite that enters the plasma because of glomerular filtration, denoted by the glomerular filtration rate (GFR). Because of the reabsorption of water, the ultimate flow of urine (urinary flow rate, $Q_u$) leaving the kidney is considerably less than the GFR. The solute present in the filtrate is also prone to reabsorption, a process that is strongly influenced by the pH of the urine and the pKa which dictate the proportion of unionized (or more lipophilic) and absorbable species. The importance of pH on drug reabsorption has been modelled (Boom et al 1994). This modelling approach readily accounts for sequential metabolism and excretion, despite the fact that both models are well-stirred and assume venous equilibration; that is, that the concentration of drug leaving the organ is the same as that in the outflow blood. In this system, the equations for the AUCs and hence the extraction ratios of precursor drug and preformed and formed metabolites have been solved for the case in which the formed metabolite is only renally excreted but is not metabolized in the isolated perfused rat kidney preparation (Geng & Pang 1999). In this situation, solutions for the AUCs of the formed and preformed metabolites differed, showing that the metabolities do not exhibit the same AUC or extraction ratio (Geng & Pang 1999). For the more complex case where the metabolite is both renally metabolized and excreted, the AUC{mi,P} and AUC{pmi} were found to differ, as also observed for E{mi,P} and E{pmi} (Table 5). It was found that precursor parameters for transport and metabolism of the precursor influenced AUC{mi,P} but not AUC{pmi}; both AUC{mi,P} and AUC{pmi} are influenced by metabolite parameters for transport and metabolism.

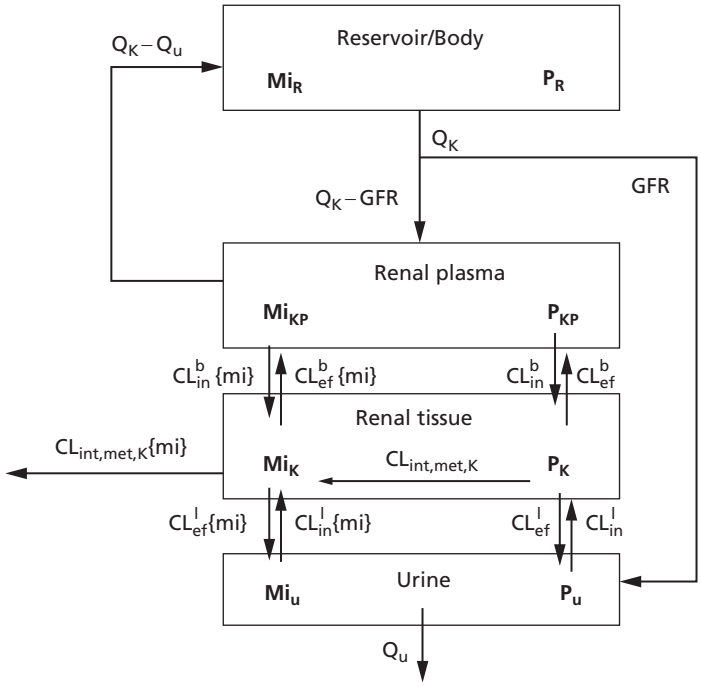


**Figure 13** A physiologically based pharmacokinetic (PBPK) model of the perfused kidney preparation. The model consists of the reservoir (R), the renal plasma (KP), renal tissue (K) and urine (u) compartments. The drug undergoes glomerular filtration (glomerular filtration rate, GFR); hence the plasma flow perfusing the kidney is the renal flow rate ($Q_K$) minus the GFR; because of water reabsorption, the returning flow to the kidney is $Q_K$ minus the urinary flow rate ($Q_u$). The influx clearances ($CL^b_{in}$ and $CL^l_{in}$) describes the entry of drug across the basolateral membrane from the blood/plasma compartment and the apical membrane of the lumen, respectively, to the kidney tissue, whereas the efflux clearances ($CL^b_{ef}$ and $CL^l_{ef}$) describe the efflux of drug from the cell to the plasma compartment (across the basolateral membrane) and into tubular urine (across the luminal membrane), respectively. Both drug precursor (P) and primary metabolite (Mi) can undergo metabolism, with intrinsic clearance ($CL_{int,met,K}$). Parameters describing Mi are further qualified by '{mi}'.

Very few studies have examined the kinetics of precursor–metabolite pairs in the kidney; even less is found on comparison of the kinetics of formed and preformed metabolites. One such study was on benzoate and its metabolite, hippurate, substrates of the monocarboxylic acid transporter (MCT) (Tamai et al 1999; Kido et al 2000) and OAT1 (Deguchi et al 2005), respectively. Upon simultaneous administration of [$^{14}$C]benzoate and [$^{3}$H]hippurate at tracer concentrations in the single-pass perfused rat kidney preparation, venous outflow and urinary concentrations of [$^{14}$C]benzoate, formed [$^{14}$C]hippurate (Figure 14A) and the preformed [$^{3}$H]hippurate (Figure 14B) were well predicted by the physiological model of the kidney (Table 6). The fitted basolateral influx and efflux clearances and reabsorptive clearances for benzoate and hippurate were similar (Table 6). However, the extraction ratios for the preformed and formed metabolite differed: E{pmi} = 0.24 and E{mi,P} = 0.39 (Geng & Pang 1999). This example is explained by a high intrinsic clearance for formation of hippurate from benzoate (40 mL min$^{-1}$ g$^{-1}$) followed by a high luminal efflux clearance (38 mL min$^{-1}$ g$^{-1}$), whereas preformed hippurate entered the tubular urine directly, bypassing the high luminal secretory clearance of hippurate (Table 6). Collectively, these events had resulted in the greater excretion of formed hippurate than preformed hippurate.

## Differences between kinetics of generated vs preformed metabolites in the intestine

Intestinal transporters and enzymes are responsible for modulating the extent of absorption of orally administered substrates (Figure 15). Apical absorptive transporters – PEPT1, apical sodium-dependent bile acid transporter (ASBT), nucleoside transporters (NTs), the OATPs and MCT1 – bring molecules into the intestinal cells, whereas apical efflux transporters (P-gp, BCRP and MRP2) are capable of returning the absorbed drug back to the lumen (Tsuji & Tamai 1996; Pang 2003). The absorbed drug that survives metabolic enzymes will eventually reach the basolateral side of intestinal cells and enter the portal circulation, sometimes utilizing transporters such as organic solute transporters (OST)$\alpha/\beta$, MRP3 or MRP4. The net process of absorption is complex, since the absorbed drug first needs to be absorbed, then it may be metabolized and effluxed, and both the drug and/or the metabolite may be re-absorbed. The drug is also subject to gastrointestinal transit and luminal degradation.

These processes have been built into the traditional physiologically based intestinal model (TM) (Figure 16A). However, intestinal metabolism is clearly route dependent: oral dosing is associated with a greater extent of intestinal

**Table 5** Equations denoting the extraction ratios of the drug or precursor (P), the preformed (pmi) and formed (mi,P) primary metabolite, based on the physiologically based model for the kidney shown in Figure 12 (Geng & Pang 1999)

| | **Equation** |
|---|---|
| AUC{pmi}<br>Mi not metabolized: | $\frac{Dose\{pmi\}\left((Q_K - Q_u)\left[CL^l_{in}\{mi\}CL^b_{ef}\{mi\} + Q_u\left(CL^b_{ef}\{mi\} + CL^l_{ef}\{mi\}\right)\right] + Q_u f_u\{mi\}CL^b_{in}\{mi\}CL^l_{ef}\{mi\}\right)}{Q_u f_u\{mi\}\left[CL^l_{ef}\{mi\}CL^b_{in}\{mi\}Q_K + \left(CL^l_{ef}\{mi\} + CL^b_{ef}\{mi\}\right)(Q_K - Q_u)GFR\right]}$ |
| Mi is metabolized: | $\frac{Dose\{pmi\}\left((Q_K - Q_u)\left[CL^l_{in}\{mi\}\left(CL^b_{ef}\{mi\} + CL_{int,met,K}\{mi\}\right) + Q_u\left(CL^b_{ef}\{mi\} + CL^l_{ef}\{mi\} + CL_{int,met,K}\{mi\}\right)\right] + f_u\{mi\}CL^b_{in}\{mi\}\left[Q_u CL^l_{ef}\{mi\} + \left(CL^l_{in}\{mi\} + Q_u\right)CL_{int,met,K}\{mi\}\right]\right)}{GFR f_u\{mi\}(Q_K - Q_u)\left[CL_{int,met,K}\{mi\}\left(CL^l_{in}\{mi\} + Q_u\right) + Q_u\left(CL^l_{ef}\{mi\} + CL^b_{ef}\{mi\}\right)\right] + Q_K f_u\{mi\}CL^b_{in}\{mi\}\left[Q_u CL^l_{ef}\{mi\} + \left(CL^l_{in}\{mi\} + Q_u\right)CL_{int,met,K}\{mi\}\right]}$ |
| AUC{mi,P}<br>Mi not metabolized,<br>Mi is metabolized: | The solutions, which are too large to be presented here, clearly show that the AUC{mi,P} depended on flow, binding, transport and intrinsic clearances pertaining to both the metabolite and the parent drug |
| *E{pmi}*<br>Mi not metabolized: | $\frac{Q_u f_u\{mi\}\left[CL^l_{ef}\{mi\}CL^b_{in}\{mi\}Q_K + \left(CL^l_{ef}\{mi\} + CL^b_{ef}\{mi\}\right)(Q_K - Q_u)GFR\right]}{Q_K\left((Q_K - Q_u)\left[CL^l_{in}\{mi\}CL^b_{ef}\{mi\} + Q_u\left(CL^b_{ef}\{mi\} + CL^l_{ef}\{mi\}\right)\right] + Q_u f_u\{mi\}CL^b_{in}\{mi\}CL^l_{ef}\{mi\}\right)}$ |
| Mi is metabolized | $\frac{GFR\, f_u\{mi\}(Q_K - Q_u)\left[CL_{int,met,K}\{mi\}\left(CL^l_{in}\{mi\} + Q_u\right) + Q_u\left(CL^l_{ef}\{mi\} + CL^b_{ef}\{mi\}\right)\right] + Q_K f_u\{mi\}CL^b_{in}\{mi\}\left[Q_u CL^l_{ef}\{mi\} + \left(CL^l_{in}\{mi\} + Q_u\right)CL_{int,met,K}\{mi\}\right]}{Q_K\left((Q_K - Q_u)\left[CL^l_{in}\{mi\}\left(CL^b_{ef}\{mi\} + CL_{int,met,K}\{mi\}\right) + Q_u\left(CL^b_{ef}\{mi\} + CL^l_{ef}\{mi\} + CL_{int,met,K}\{mi\}\right)\right] + f_u\{mi\}CL^b_{in}\{mi\}\left[Q_u CL^l_{ef}\{mi\} + \left(CL^l_{in}\{mi\} + Q_u\right)CL_{int,met,K}\{mi\}\right]\right)}$ |
| *E{mi,P}*<br>Mi not metabolized | $\frac{CL^l_{ef}\{mi\}Q_u\left(f_u\{mi\}CL^b_{in}\{mi\} + (Q_K - Q_u)\right)}{Q_u f_u\{mi\}CL^b_{in}\{mi\}CL^l_{ef}\{mi\} + (Q_K - Q_u)\left[\left(CL^l_{in}\{mi\} + Q_u\right)CL^b_{ef}\{mi\} + CL^l_{ef}\{mi\}Q_u\right]}$ |
| Mi is metabolized | $\frac{\left[Q_u CL^l_{ef}\{mi\} + \left(CL^l_{in}\{mi\} + Q_u\right)CL_{int,met,K}\{mi\}\right]\left[f_u\{mi\}CL^b_{in}\{mi\} + (Q_K - Q_u)\right]}{f_u\{mi\}CL^b_{in}\{mi\}\left[\left(CL^l_{in}\{mi\} + Q_u\right)CL_{int,met,K}\{mi\} + CL^l_{ef}\{mi\}Q_u\right] + (Q_K - Q_u)\left[\left(CL^l_{in}\{mi\} + Q_u\right)CL^b_{ef}\{mi\} + CL^l_{ef}\{mi\}Q_u + \left(CL^l_{in}\{mi\} + Q_u\right)CL_{int,met,K}\{mi\}\right]}$ |
| *E{pmi}/E{mi,P}*<br>Mi not metabolized | $\frac{f_u\{mi\}\left[CL^l_{ef}\{mi\}CL^b_{in}\{mi\}Q_K + \left(CL^l_{ef}\{mi\} + CL^b_{ef}\{mi\}\right)(Q_K - Q_u)GFR\right]}{Q_K CL^l_{ef}\{mi\}Q_u\left(f_u\{mi\}CL^b_{in}\{mi\} + (Q_K - Q_u)\right)}$ |
| Mi is metabolized | $\frac{GFR\, f_u\{mi\}(Q_K - Q_u)\left[CL_{int,met,K}\{mi\}\left(CL^l_{in}\{mi\} + Q_u\right) + Q_u\left(CL^l_{ef}\{mi\} + CL^b_{ef}\{mi\}\right)\right] + Q_K f_u\{mi\}CL^b_{in}\{mi\}\left[Q_u CL^l_{ef}\{mi\} + \left(CL^l_{in}\{mi\} + Q_u\right)CL_{int,met,K}\{mi\}\right]}{Q_K\left[Q_u CL^l_{ef}\{mi\} + \left(CL^l_{in}\{mi\} + Q_u\right)CL_{int,met,K}\{mi\}\right]\left[f_u\{mi\}CL^b_{in}\{mi\} + (Q_K - Q_u)\right]}$ |

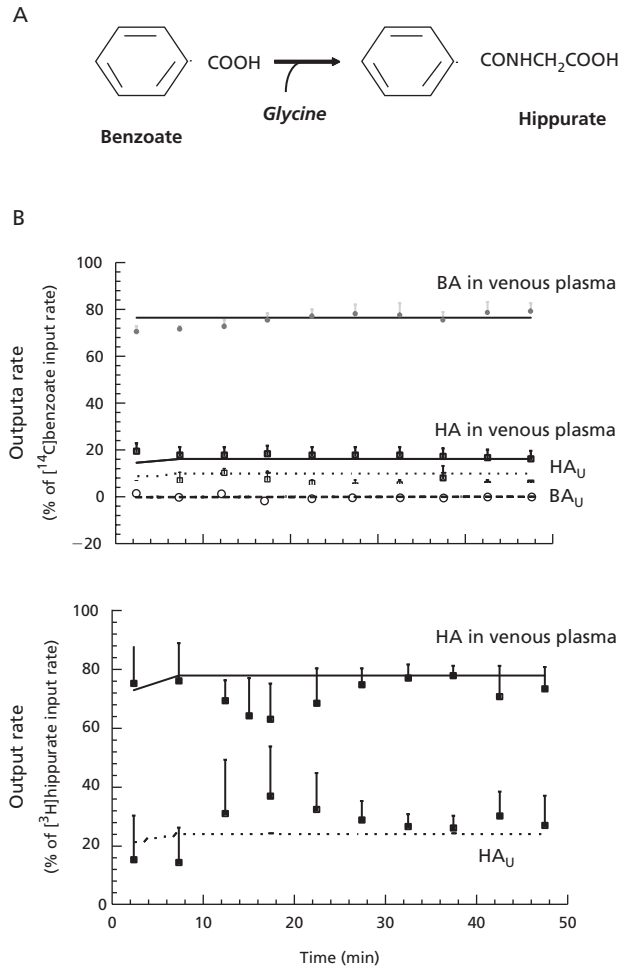


**Figure 14** A. Schematic depiction of the metabolism of benzoic acid (BA) to hippuric acid (HA) by glycine conjugation. B. Output rates of BA and HA in outflow perfusate and urine after single-pass rat kidney perfusion with [$^{14}$C]benzoate (upper graph), or after giving preformed [$^{3}$H]HA (lower graph) simultaneously into the single-pass perfused rat kidney. The lines are predictions from the physiologically based pharmacokinetic model depicted in Figure 13. (Taken from Geng & Pang (1999), with permission.)

**Table 6** Parameters for the handling of benzoic acid and hippuric acid obtained from fitting to the physiological model in rat kidney (Figure 14, from Geng & Pang 1999)

| **Parameters obtained from fits to the physiologically based model for kidney** | **mL min$^{-1}$ g$^{-1}$** |
|---|---|
| **Optimized clearances for benzoic acid** | |
| Metabolic intrinsic clearance: $CL_{int,met,K}$ | 40 |
| Basolateral influx clearance: $CL^{b}_{in}$ | 13 |
| Basolateral efflux clearance: $CL^{b}_{ef}$ | 2.5 |
| Luminal efflux clearance: $CL^{l}_{ef}$ | 10 |
| Luminal reabsorption clearance: $CL^{l}_{in}$ | 3 |
| **Optimized clearances for hippuric acid** | |
| Basolateral influx clearance: $CL^{b}_{in}\{mi\}$ | 14 |
| Basolateral efflux clearance: $CL^{b}_{ef}\{mi\}$ | 4 |
| Luminal efflux clearance: $CL^{l}_{ef}\{mi\}$ | 38 |
| Luminal reabsorption clearance: $CL^{l}_{in}\{mi\}$ | 4 |

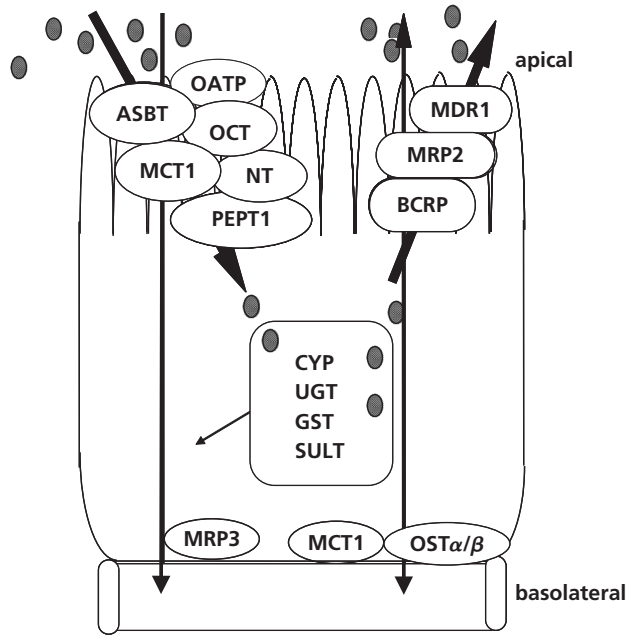


**Figure 15** Depiction of transporters at the apical membrane of enterocytes for absorption and efflux, and at the basolateral membrane for entry into the circulation (Taken from Pang (2003), with permission). Definitions of transporter abbreviations are given in the glossary. NT, nucleoside transporter; SULT, sulfotransferase.

metabolism than with systemic dosing (Doherty & Pang 2000); some of these seminal studies were conducted in the perfused rat intestine preparation, and some were observations in man (Table 7). To account for this phenomenon, the segregated flow model (SFM) was developed (Cong et al 2000), which is similar to that described by Klippert & Noordhoek (1985) (Figure 16B). This model views intestinal flow as segregated: a small minor flow perfuses an active subcompartment containing the enterocytes which mediate absorption, metabolism and efflux, whereas a much larger flow perfuses the storage subcompartment, representing the remaining intestinal tissue which contains the serosal region and part of the submucosa and mucosa.

The rate equations for the AUCs of the preformed metabolite {pmi} and formed primary metabolite {mi,P} after i.v. and oral administration of drug/preformed metabolite have been solved in the TM and SFM (Sun & Pang 2008). The results showed marked similarity in the $AUC_{iv}\{pmi\}$ between the two models. For the TM, the flow rate was the total intestinal flow ($Q_I$), whereas for SFM, the flow rate in the equation was the enterocyte flow rate ($Q_{en}$) (Table 8). For this reason, the availability of the preformed metabolite differed only with $Q_I$ and $Q_{en}$ in the formula. There was no difference in the $AUC_{po}\{pmi\}$, $AUC_{po}\{mi,P\}$ or the $AUC_{iv}\{mi,P\}$ between the TM and SFM, since the flow terms disappeared. However, it is clear from the solutions that the fate of the formed metabolite (shown by the AUC{mi,P}) differed from that of the preformed metabolite (AUC{pmi}) for both TM and SFM. The difference was route dependent (oral vs i.v.) and flow-pattern dependent (TM vs SFM) (Table 8). AUC{mi,P} was found to be dependent on drug parameters, whereas

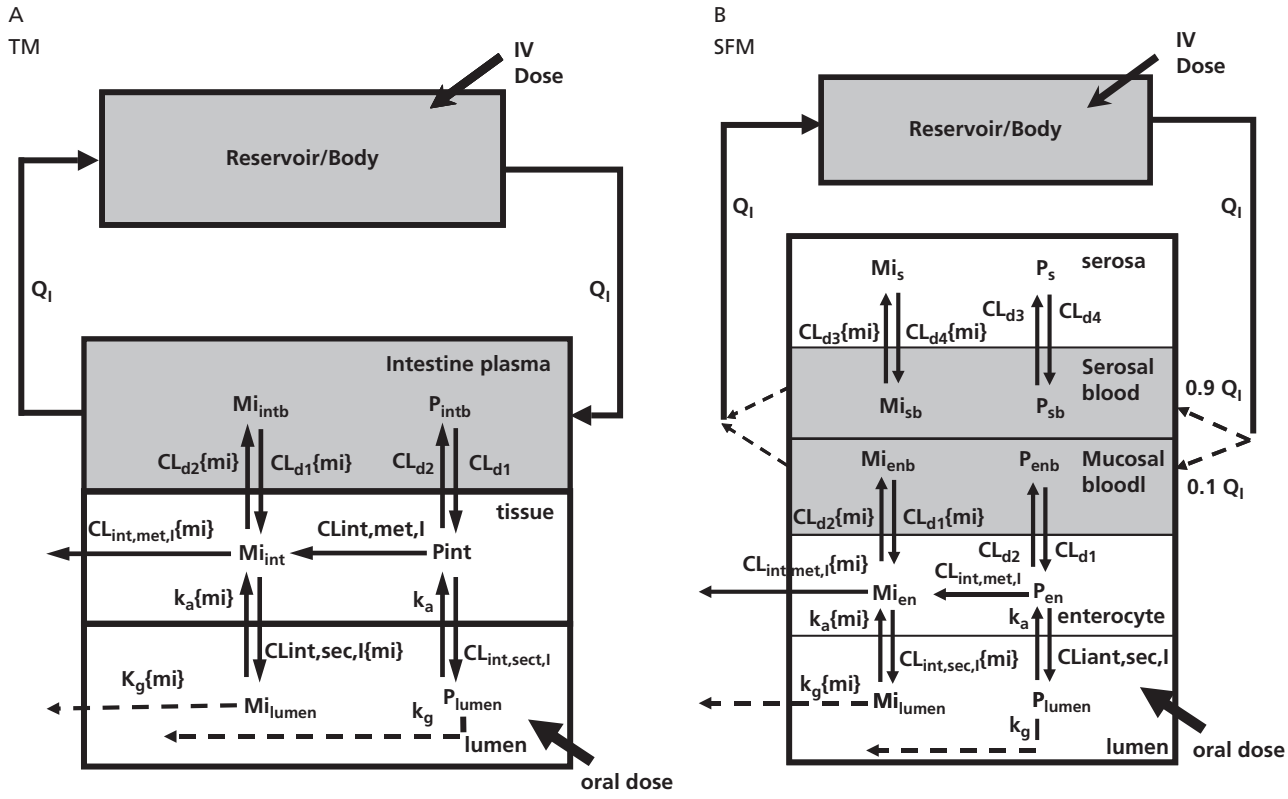


**Figure 16** Depiction of the traditional model (A) and the segregated flow model (B) that describe drug and metabolite metabolism and secretion in the vascularly perfused small intestine preparation. Definitions of abbreviations are given in the glossary; $C_{Ld3}$ is the drug influx and influx clearance across the seroal membrane from blood; $CL_{d4}$ is the efflux clearance from the serosal compartment to blood; $k_g$ is the degradation rate constant in the lumen due to gastrointestinal transit or metabolism.

AUC{pmi} was not. However, the $AUC_{po}\{mi,P\}/AUC_{iv}\{mi,P\}$ resulting from drug administration yields meaningful information as to the fraction of precursor drug absorbed ($F_{abs}$). The relationship pertains to the case where the metabolite is formed only in the intestine and not elsewhere (Sun & Pang 2008).

Varying segmental distributions of both enzymes and transporters have been found within the small intestine (Pang 2003). Generally speaking, most of the enzymes are more abundant in the duodenal or proximal region of the small intestine (Table 9). The distribution of absorptive transporters may be proximal (PEPT1), jejunal (MRP2, NTs) or distal (ASBT and MRP3). Accordingly, the TM and SFM models can be expanded into the segmental traditional model (STM) and the segmental segregated flow model (SSFM) to include the heterogeneous distributions of transporters and enzymes within the three segments of the small intestine (Tam et al 2003). The heterogeneous distribution of transporters and enzymes is shown to influence drug bioavailability on the basis of simulations from the STM and SSFM. The most important factor was found to be the distribution of enzymes along the intestine (Tam et al 2003). According to the STM

**Table 7** Examples of drugs that display route-dependent intestinal metabolism

| Drugs | Appearance of metabolite | | References |
|---|---|---|---|
| | Intraduodenal administration | Vascular administration | |
| Acetaminophen | Glucuronide/sulfate | Not observed | Pang et al 1986 |
| Enalapril | Enalaprilat | Not observed | Pang et al 1985b |
| Cyclosporin | + | Not observed | Ducharme et al 1995 |
| Tacrolimus | + | Not observed | Lampen et al 1995 |
| Midazolam | ++ | Less compared with luminal administration | Paine et al 1996, 1997; Thummel et al 1996 |
| Aminocarbovir | Carbovir | Less compared with luminal administration | Wen et al 1999 |
| Morphine | 3$\beta$-Glucuronide | Not observed | Doherty et al 2007 |

+ denotes 'more'observed compared with i.v. or vascular administration; ++ denotes 'much more'observed compared with i.v. or vascular administration.

**Table 8** Equations for the area under the curve (AUC) for preformed {pmi}and formed {mi,P} metabolites according to the traditional model (TM) and segregated flow model (SFM), with different routes of administration (134)

| Parameter | Equations |
|---|---|
| $AUC_{iv}\{pmi\}$ for TM | $\frac{Dose_{iv}\{pmi\}\left[CL_{int,sec,I}\{mi\}(Q_I + CL_{d1}\{mi\})(1 - F_{abs}\{mi\}) + Q_I\left(CL_{d2}\{mi\} + CL_{int,met,I}\{mi\}\right) + CL_{d1}\{mi\}CL_{int,met,I}\{mi\}\right]}{Q_I CL_{d1}\{mi\}\left(CL_{int,sec,I}\{mi\}\left(1 - F_{abs}\{mi\}\right) + CL_{int,met,I}\{mi\}\right)}$ |
| $AUC_{iv}\{pmi\}$ for SFM | $\frac{Dose_{iv}\{pmi\}\left[CL_{int,sec,I}\{mi\}(Q_{en} + CL_{d1}\{mi\})(1 - F_{abs}\{mi\}) + Q_{en}\left(CL_{d2}\{mi\} + CL_{int,met,I}\{mi\}\right) + CL_{d1}\{mi\}CL_{int,met,I}\{mi\}\right]}{Q_{en} CL_{d1}\{mi\}\left(CL_{int,sec,I}\{mi\}\left(1 - F_{abs}\{mi\}\right) + CL_{int,met,I}\{mi\}\right)}$ |
| $AUC_{po}\{pmi\}$ for TM & SFM | $\frac{Dose_{po}\{pmi\}F_{abs}\{mi\}CL_{d2}\{mi\}}{CL_{d1}\{mi\}\left(CL_{int,sec,I}\{mi\}\left(1 - F_{abs}\{mi\}\right) + CL_{int,met,I}\{mi\}\right)}$ |
| $AUC_{iv}\{mi,P\}$ for TM & SFM | $\frac{Dose_{iv}CL_{int,met,I}CL_{d2}\{mi\}}{CL_{d1}\{mi\}\left(CL_{int,sec,I}\{mi\}(1 - F_{abs}\{mi\}) + CL_{int,met,I}\{mi\}\right)((1 - F_{abs})CL_{int,sec,I} + CL_{int,met,I})}$ |
| $AUC_{po}\{mi,P\}$ for TM & SFM | $\frac{Dose_{po}F_{abs}CL_{int,met,I}CL_{d2}\{mi\}}{CL_{d1}\{mi\}\left(CL_{int,sec,I}\{mi\}(1 - F_{abs}\{mi\}) + CL_{int,met,I}\{mi\}\right)((1 - F_{abs})CL_{int,sec,I} + CL_{int,met,I})}$ |
| $\frac{AUC_{po}\{mi,P\}}{AUC_{iv}\{mi,P\}}$ for TM & SFM | $\frac{F_{abs}Dose_{po}}{Dose_{iv}}$ |
| $\frac{AUC_{po}\{mi,P\}}{AUC_{po}\{pmi\}}$ for TM & SFM | $\frac{Dose_{po}F_{abs}CL_{int,met,I}}{Dose_{po}\{pmi\}F_{abs}\{mi\}(CL_{int,sec}(1 - F_{abs}) + CL_{int,met,I})}$ |
| $\frac{AUC_{iv}\{mi,P\}}{AUC_{iv}\{pmi\}}$ for TM | $\frac{Dose_{iv}Q_I CL_{int,met,I}CL_{d2}\{mi\}}{Dose_{iv}\{pmi\}((1 - F_{abs})CL_{int,sec,I} + CL_{int,met,I})\left(CL_{int,sec,I}\{mi\}(CL_{d1}\{mi\} + Q_I)(1 - F_{abs}\{mi\}) + Q_I\left(CL_{d2}\{mi\} + CL_{int,met,I}\{mi\}\right) + CL_{d1}\{mi\}CL_{int,met,I}\{mi\}\right)}$ |
| $\frac{AUC_{po}\{pmi\}}{AUC_{iv}\{pmi\}}$ for TM | $\frac{F_{abs}\{mi\}Dose_{po}\{pmi\}Q_I CL_{d2}\{mi\}}{Dose_{iv}\{pmi\}\left[CL_{int,sec,I}\{mi\}(Q_I + CL_{d1}\{mi\})(1 - F_{abs}\{mi\}) + Q_I\left(CL_{d2}\{mi\} + CL_{int,met,I}\{mi\}\right) + CL_{d1}\{mi\}CL_{int,met,I}\{mi\}\right]}$ |
| $\frac{AUC_{iv}\{mi,P\}}{AUC_{iv}\{pmi\}}$ for SFM | $\frac{Dose_{iv}Q_{en}CL_{int,met,I}CL_{d2}\{mi\}}{Dose_{iv}\{pmi\}((1 - F_{abs})CL_{int,sec,I} + CL_{int,met,I})\left(CL_{int,sec,I}\{mi\}(CL_{d1}\{mi\} + Q_{en})(1 - F_{abs}\{mi\}) + Q_{en}\left(CL_{d2}\{mi\} + CL_{int,met,I}\{mi\}\right) + CL_{d1}\{mi\}CL_{int,met,I}\{mi\}\right)}$ |
| $\frac{AUC_{po}\{pmi\}}{AUC_{iv}\{pmi\}}$ for SFM | $\frac{F_{abs}\{mi\}Dose_{po}\{pmi\}Q_{en}CL_{d2}\{mi\}}{Dose_{iv}\{pmi\}\left[CL_{int,sec,I}\{mi\}(Q_{en} + CL_{d1}\{mi\})(1 - F_{abs}\{mi\}) + Q_{en}\left(CL_{d2}\{mi\} + CL_{int,met,I}\{mi\}\right) + CL_{d1}\{mi\}CL_{int,met,I}\{mi\}\right]}$ |

[a] $F_{abs} = \frac{k_a}{k_a + k_g}$ and $F_{abs}\{mi\} = \frac{k_a\{mi\}}{k_a\{mi\} + k_g\{mi\}}$.

**Table 9** Segmental distribution of transporters and enzymes in the small intestine and colon in animals and humans (based on protein or mRNA)

| Transporter/enzyme | Distribution patterns in intestinal segments | References |
|---|---|---|
| Cyp3a | duodenum > jejunum > ileum | Hoensch et al 1975; Dubey & Singh 1988a; Paine et al 1996; 1997; Li et al 2002; Liu et al 2006 |
| Ugt | duodenum > jejunum > ileum | Koster et al 1985; Dubey & Singh 1988b |
| Sult | duodenum > jejunum > ileum | Schwarz & Schwenk 1984 |
| Gst | duodenum ~ jejunum > ileum | Pinkus et al 1997 |
| PEPT1 | proximal intestine > distal ileum | Fei et al 1994 |
| ASBT | distal intestine > proximal intestine | Wong et al 1994; Shneider et al 1995; Aldini et al 1996; Chen et al 2006a |
| Mct1 | duodenum < jejunum > ileum | Tamai et al 1999; Cong et al 2001; Gill et al 2005; Englund et al 2006 |
| Nt | highest in jejunum | Ngo et al 2001 |
| Mrp2 | duodenum = jejunum > ileum | Gotoh et al 2000; Mottino et al 2000 |
| P-glycoprotein | ileum > jejunum > duodenum | Li et al 2002; Chen et al 2006b |
| Oatp3 | highest in jejunum | Walters et al 2000 |
| Mrp3 | ileum> jejunum > duodenum | Rost et al 2002 |

Definitions and abbreviations are given in the glossary.

and the SSFM, the role of efflux transporters such as P-gp may not be significant. First, P-gp is distally distributed in the small intestine, and drug absorption may already be completed in the proximal segments, where P-gp expression is low. Second, drug absorption is not sensitive to P-gp, and this can be rationalized by the ease of reabsorption of the effluxed substrates because of their lipophilicity/high permeability. Hence, there is truth in the statement that the role of P-gp is over-emphasized in deterring absorption (Lin et al 1999). Because of these transporters, drugs are found to be absorbed in various proportions in different regions of the intestine (Gramatté 1996; Gramatté et al 1994, 1996). Knowledge on the transporters has allowed the judicious design of prodrugs that will utilize transporters for absorption, thus improving their bioavailability (Table 10). Needless to say, the conditions must include the biopharmaceutical and physicochemical data that govern the dissolution of the prodrug and the drug. Factors such as structural diversity, excipients and dissolution properties will also need to be considered for drug absorption.

## Other considerations

### *Precursor influence due to 'distributed-in-space' behaviour*

The types of theoretical modelling approaches described for the liver, kidney and intestine have failed to take into consideration the fact that drug and metabolite processing in eliminating organs is a distributed-in-space phenomenon – that the kinetic profiles should be related to the space and time domains that describe the influence of the precursor species on the kinetics of formed metabolites (Goresky et al 1993a, b; Pang et al 1998b; Schwab & Pang 2000). Both the compartmental and physiological models consider the tissue as a single well-stirred compartment, precluding the notion that drug characteristics will influence the handling and sequential removal of the metabolite. These factors currently remain unaccounted for in whole-body pharmacokinetics. In the liver, however, the parallel-tube model and the dispersion models that have emerged to describe drug clearance are more capable of accounting for distributed-in-space behaviour in organ processing (Goresky et al 1993a, b; Pang et al 1998; Schwab & Pang 2000). These models are more apt to relate to the influence of precursor characteristics on successive formation of metabolites and their kinetics (Pang 1995). Again, because of their complexity, these types of modelling for the whole body are seldom explored.

### *Excretion clearance of formed metabolites*

Formed and preformed metabolites display different excretory clearance profiles within the formation organ (Figure 17). The excreted metabolite originates partially from the recirculating existing or preformed metabolite species and partially from immediate excretion of the formed metabolite. As long as drug is present and continues to form the metabolite, this second component, which is time dependent, persists. When the drug is no longer present, the component becomes zero, and the excretion clearance of the formed metabolite will now approach that of the preformed species. The trend of a descending clearance profile of the formed species with time indicates that the organ examined is a metabolite formation organ. The phenomenon had been explained by de Lannoy and Pang in both the liver and kidney for enalaprilat, as a preformed species and species formed from enalapril (de Lannoy & Pang 1993; de Lannoy et al 1993).

In a further comparison, de Lannoy and Pang showed that, with the presence of more metabolite formation organs (LK for liver and kidney, vs L or K for liver or kidney alone) will result in higher metabolite levels in the circulation (de Lannoy & Pang 1993). The presence of multiple organs changed not only the metabolite profiles in blood but also the observed excretory clearance of the formed metabolite, by altering the ratio of preformed metabolite in circulation and the formed metabolite within formation organs (Figure 17). In addition, a change in the volume of distribution of the metabolite (shown as LK vs LK2, where LK2 represents a doubling of the volume compared with LK in the combined liver–kidney perfusion systems) further affected the kinetics

**Table 10** Prodrugs that utilize transporters to enhance intestinal absorption or uptake into cells

| Prodrug | Transporter | Active moiety | References |
|---|---|---|---|
| Alafosfalin (l-alanyl-L-1-aminoethyl-phosphonic acid) | PEPT1 | Aminoethylphosphonic acid | Grappel et al 1985 |
| Fosinopril | PEPT1 | Fosinoprilat | Friedman & Amidon 1989 |
| L-Valyl ester of zidovudine | PEPT1 | Zidovudine | Han et al 1988 |
| Pro-phe-pamidronate, pro-phe-alendronate | PEPT1 | Pamidronate, alendronate | Ezra et al 2000 |
| Valacyclovir | PEPT1 | Acyclovir | Friedrichsen et al 2002 |
| Valganciclovir | PEPT1 | Ganciclovir | Brown et al 1999 |
| Valtorcitabine (NM283) (L-valine ester prodrug) | | 2′-C-Methylcytidine (NM107) | Pierra et al 2006 |
| Talaglumetad (LY544344, L-alanylamide prodrug) | PEPT1 | (+)-2-Aminobicyclo [3.1.0]hexane-2,6-dicarboxylic acid (LY354740) | Bueno et al 2005 |
| L-DOPA | Amino acid transporter | Dopamine | Shindo et al 1973; Wade et al 1973; Tsuji 1999 |
| L-α-Methyldopa | Amino acid transporter | α-Methylnorepinephrine | Robertson et al 1984 |
| Thiovir | Amino acid transporter | Foscarnet | Waninger et al 2007 |
| D-MOD-L-Glu | Amino acid transporter | p-Di(hydroxyethyl)-amino-D-phenylalanine (D-MOD) | Sakaeda et al 2000 |
| Ganciclovir-glutamate monoester | Amino acid transporter | Ganciclovir | Gandhi et al 2005 |
| Carindacillin | MCT1 | Carbenicillin | Li et al 1999 |
| XP13512 | MCT1 | Gabapentin | Cundy et al 2004 |
| L-Dehydroascorbic acid | GLUT1 | L-Ascorbic acid | Agus et al 2007 |
| L-Serinyl-b-D-glucoside analogues of met$^5$enkephalin | GLUT1 | Met$^5$enkephalin | Polt et al 1994 |
| Zidovudine (AZT ) | ENT | 3′-Azido-3′-deoxythymidine (AZT) triphosphate | Sato et al 2007 |
| Capecitabine | hCNT1 | 5-Fluorouracil | Desmoulin et al 2002 |
| Amino acid ester prodrugs including Val, Phe, Pro, Asp and Lys esters | hCNT2 | 5,6-Dichloro-2-bromo-1-β-D-ribofuranosylbenzimidazole (BDCRB) | Shin et al 2006 |
| Lovastatin and HR 780 conjugated with bile acids | NTCP | Lovastatin and HR 780, HMGCoA reductase inhibitors (statins) | Petzinger et al 1995 |
| Abacavir | ASBT | Carbovir | Majumdar et al 2004 |
| Chlorambucil-taurocholic acid | ASBT | Chlorambucil | Kullak-Ublick et al 1997 |
| Fosfomycin | NPT | | Ishizawa et al 1992 |
| Lisdexamfetamine | Unknown | D-Amphetamine | Blick & Keating 2007 |
| Betamethasone | Unknown | Acetate or phosphate | Samtani et al 2005 |
| Irinotecan | OATP1B1 (OATP-C) | 7-Ethyl-10-hydroxycamptothecin (SN-38) | Nozawa et al 2005 |
| Ester prodrug tenofovir disoproxil fumarate | Unknown | Tenofovir | van Gelder et al 2002 |
| Ascorbic acid conjugated diclofenac | Ascorbic acid transporter 2 (SVCT2) | Diclofenac | Dalpiaz et al 2005 |

Definitions of abbreviations are given in the glossary.

of the formed metabolite. The volume of the metabolite would alter the concentration of the preformed species, again changing the ratio of preformed:formed metabolite in the system (de Lannoy & Pang 1993; de Lannoy et al 1993).

### Extrapolation of these concepts from animals to man

The extrapolation of these concepts derived for single organs to the whole body is similar to that used in the development of physiologically based pharmacokinetic (PBPK) models for the whole body. The strategy is to use in-vitro data on binding, metabolism, transport and tissue partitioning, together with flow and volumes, to build the PBPK model for the whole body. However, the scale up of the PBPK model from animals to man necessitates use of blood flows and volumes pertaining to man, whereas tissue partition coefficients found in animals may be used unless the outcome shows poor predictions. However, the metabolic constants ($K_m$ and $V_{max}$) must be investigated in human tissues to avoid the complication of species differences. Since additional changes are anticipated when transporters are involved, it would be prudent to conduct transport studies with human hepatocytes (Shitara et al 2003). There have been many successes in scaling the physiological model based on animal data and in-vitro human data on the parent drug to man (Kawai et al 1988; Himmelstein et al 2004; Parrott et al 2005).

The next question is how to utilize the concepts developed in modelling of the liver, kidney and intestine in this review to

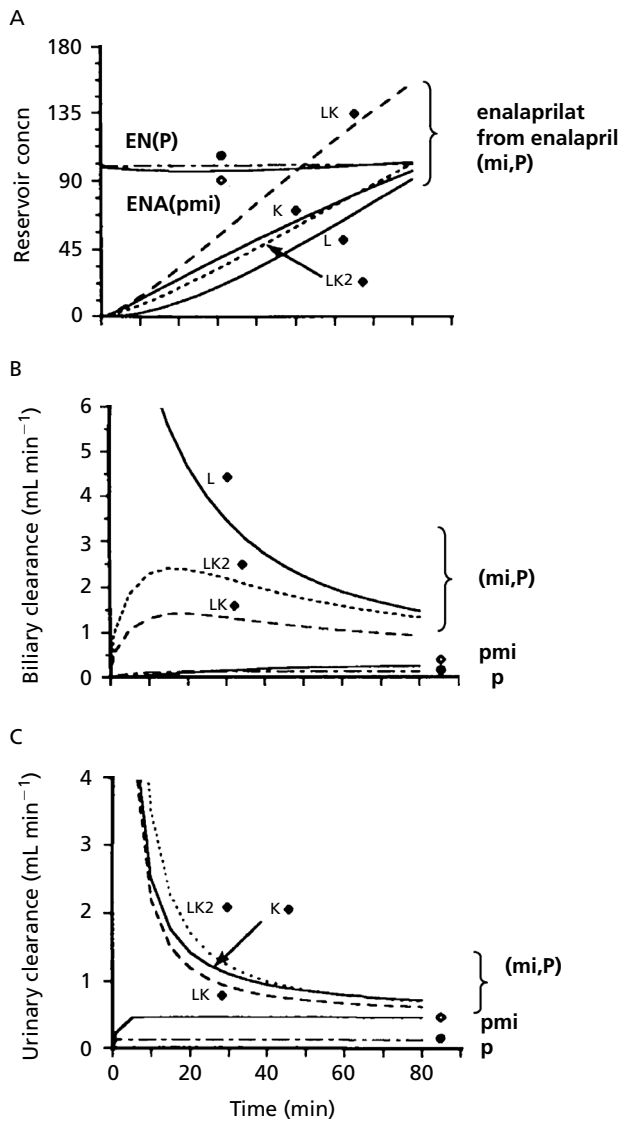


**Figure 17** A. Metabolite formation organs and volume affect the profiles for the generated metabolite (mi,P), enalaprilat. Predictions (lines) on the kidney (K), the liver (L), LK (L and K are both metabolite formation organs) and LK2 (when the volume of the reservoir was doubled) were simulated on the basis of data obtained from de Lannoy & Pang (1993) on the liver and kidney perfusion experiments. Changing the number of formation organs or the volume did not affect the profiles of the precursor, enalapril [EN(P)], nor the preformed metabolite (enalaprilat; ENA(pmi)). The number of organs and the volume further affected the apparent biliary clearance (B) and the urinary clearance (C), but not those for the preformed species, P and pmi. (Taken from de Lannoy & Pang (1993), with permission.)

extend these to humans. It is rare that metabolites are measured and modelled, and it is rarer that this kind of information is known for the metabolite. The strategy so far has been physiological modelling of one organ at a time. If indeed the liver is the only organ involved, then the model developed for the liver only would be useful, as long as blood flows and volumes are scaled up from animal to man, and when data on binding, transport and metabolism in human hepatocytes are available for scaled-up input into the model. Other non-eliminating organs may be lumped as highly perfused and poorly perfused tissues (Nestorov et al 1998). However, the interaction of multiple organs of metabolism has not been examined systematically. The intestine or renal model may be added to that of the liver PBPK model. In order to make good predictions in-vivo, we need to know where the metabolite is formed and how the metabolite is further handled (organ and mechanism of metabolism or excretion). Unfortunately, many data are needed to test these models on metabolite kinetics in man. The factor that has not been mentioned so far is binding to vascular and tissue proteins, and this aspect can be modelled as well (Pang et al 1995; Liu et al 2005). If all these factors are known, then it is possible to render the models to be predictive of metabolite disposition in man. We surmise that metabolite AUC{mi,P} will change case by case, depending on which organs/tissues are involved in the formation and handling, and both drug and metabolite characteristics.

## Concluding remarks

Metabolite kinetics constitutes an important area of study. The most important question is whether the metabolite contributes to toxicity. In many instances, drugs/metabolites may interact with a receptor or enzyme to elicit cardiotoxicity or to form reactive metabolites that result in covalent binding of protein or DNA to disrupt function. There is the need to investigate the toxic potential of metabolites. One thought is to ignore metabolites if these are found in small quantities in excreta or blood. However, this would not consider the scenarios of some toxic metabolites that are present only in minute quantities or could not be detected. Another is to administer metabolites to investigate the toxicities in either animal or human studies. However, as suggested by many observations and in this review, good correspondence may not be found. The dilemma therefore remains.

As shown from this review, metabolites formed from precursor drugs may behave differently from administered preformed metabolite species, unless the drug and metabolite are of high permeability and are independent of transporter-mediated transport, and that enzymes involved in metabolite formation are readily accessible. Clearly, if any of these assumptions is violated, the preformed metabolite will not behave in the same fashion as the formed metabolite. Then what kind of information is forthcoming from administration of preformed metabolites? Can some sound strategies be formulated?

The key issue is to recognize that comparable toxicity data may not be provided by administration of the preformed metabolite, since the kinetics and exposure of preformed metabolites can indeed differ from the kinetics of metabolite formed after administration of the parent drug. Although the metabolite study may not provide firm data on toxicity, the information provided can affirm or forecast the type of model that is needed for building the model in animals and for scale up to humans. There have been proposals to quantify metabolite formation in terms of clearance estimates, as the fractional total body clearance ($g_{mi}$), or effective formation clearance ($f_{mi}$) as commented by Pang & Kwan (1983), rather than using quantities or % AUC. Background

information as to which are organs for formation and further elimination, as well as the enzymes and types of transporters involved, and which are the compensatory pathways (Morris & Pang 1987; Sirianni & Pang 1997; Chang et al 2006) would serve as the building blocks for physiological models. Upon correction for binding (Halifax & Houston 2006), hepatic microsomes or cytosol, recombinant enzyme systems or cryopreserved hepatocytes (Obach 1999; Soars et al 2002; McGinnity et al 2004; Ito & Houston 2004, 2005; Riley et al 2005; Joulin et al 2006) prepared from both animals species used in preclinical studies and humans may be used to predict in-vivo clearances. Scaling factors could be used to translate in-vitro data to in-vivo and animal scaling to man may then be performed (Boxenbaum 1982; Boxenbaum & Ronfeld 1983). The data from humans on transport and metabolism could be acquired in cell expression systems of transporters with the relative activity factor method and in hepatocytes (Shitara et al 2003; Barter et al 2007; Somers et al 2007; Yamada et al 2007). Information should be obtained from in-vitro studies on the toxic pathway resulting from drug administration, permeation and distribution of drug/metabolite into the organ/tissue, metabolism within, efflux and handling of the formed metabolite. All of the above information should be aptly incorporated into a useful PBPK model of precursor drug and metabolite for predictions. The facile use of commercially available software (such as the Gastroplus) and the PBPK model can lead to reasonable predictions of human pharmacokinetics and play a critical role in risk assessment (Sawada et al 1985; Nestorov et al 1998; Poulin & Theil 2002a, b; Jones et al 2006; De Buck et al 2007). Refinement of the models and sound predictions of risks and toxicity may be improved through modelling and simulations.

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## Glossary for kinetic parameters

| | |
|---|---|
| P, Mi, Mii | precursor and primary and secondary metabolites, respectively |
| {pmi} | qualifying the preformed primary metabolite |
| {mi,P} | qualifying the primary metabolite formed from precursor drug |
| {mii,mi} | qualifying the secondary metabolite formed from primary metabolite |
| {mii,P} | qualifying the secondary metabolite resulting from sequential metabolism of the precursor |
| k | elimination rate constant of precursor drug |
| $k_e$, $k_e${mi}, $k_e${mii} | excretion rate constants of precursor drug and primary (mi) and secondary (mii) metabolites, respectively |
| $k_{mi}$ | formation rate constant of the primary metabolite, Mi |
| $k_{fmi}$ | effective metabolite formation rate constant |
| $k_{m1}$, $k_{m2}$, $k_{m3}$ | formation rate constants of primary metabolites M1, M2 and M3 from the precursor drug, respectively |
| $k_m${mi}, k{mi} | metabolic and elimination rate constants of mi, respectively |
| $k_m$ | metabolite formation rate constant in-vitro |
| F{mi,P}, F{pmi} | available fractions of formed and preformed primary metabolites, respectively |
| E{mi,P}, E{pmi} | extraction ratios of formed and preformed primary metabolites, respectively |
| V, V{mi} | volumes of distribution of precursor and primary metabolite, respectively |
| $CL_{in}$, $CL_{ef}$ | influx and efflux clearances, respectively |
| $CL_{int,met}$, $CL_{int,sec}$ | metabolic intrinsic and excretory intrinsic clearances, respectively |
| Q | flow rate |

**Subscripts**

| | |
|---|---|
| L and K | qualifying the intestine and kidney, respectively |
| u | urine |
| R, LB, L | reservoir, liver blood, and liver tissue, respectively |
| intb, int, lumen | intestinal blood, intestine tissue and lumen, respectively |
| s, sb, enb, en | serosa, serosal blood, enterocyte and mucosal blood or enterocyte, respectively |

## Glossary for transporters and enzymes

| | |
|---|---|
| ASBT | Apical sodium-dependent bile acid transporter, SLC10A2 |
| BCRP | Breast cancer resistance protein, ABCG2 |
| BSEP | Bile salt export pump, ABCB11 |
| CNT1,2 | Concentrative nucleoside transporter 1 and 2, SLC28A1–2 |
| CYP | Cytochrome P450 |
| GLUT1 | Glucose transporter 1, SLC2A1 |
| GST | Glutathione S-transferase |
| MCT1 | Monocarboxylic acid transporter 1, SLC16A1 |
| MDR1 | Multidrug resistance protein 1, ABCB1 |
| MDR3 | Multidrug resistance protein 3, ABCB4 |
| MRP2–6 | Multidrug resistance-associated protein isoforms 2–6, ABCC2–6 |

| | |
|---|---|
| NT | Nucleoside transporter |
| NTCP | Sodium-dependent taurocholate cotransporting polypeptide, SLC10A1 |
| OAT 1–3 | Organic anion transporters 1–3, SLC22A6-8 |
| OCT 1–3 | Organic cation transporters 1–3, SLC22A1–3 |
| OCTN2 | Organic cation/carnitine transporter, SLC22A5 |
| OATP | Organic anion cotransporting polypeptide, SLCO |
| OST$\alpha$/$\beta$ | Organic solute transporters $\alpha$ and $\beta$ |
| PEPT1, PETP2 | Oligopeptide transporters 1 and 2, SLC15A1–2 |
| SULT | Sulfotransferases |
| SULT1A1 | Phenolic sulfotransferase for planar compounds, also known as PST |
| SULT2A1 | Hydroxysteroid sulfotransferase |
| SULT1E1 | Estrogen sulfotransferase |
| UGT | UDP-glucuronosyltransferase |