Physicochemical and drug-delivery considerations for oral drug bioavailability

O. Helen Chan and Barbra H. Stewart

The bioavailability of a compound after oral administration is a function of molecular characteristics, dosage form design, and the barrier functions of the organism. The extent of intestinal absorption is dependent on drug stability, solubility and permeability. First-pass elimination can be effected by intestinal mucosal or hepatic processing. Biological availability is dependent on the physicochemical parameters of the drug, including molecular weight and lipophilicity, as well as on specific structural features such as H-bonding capacity. This review illustrates the relationships between physicochemical parameters, intestinal permeability and hepatic processing, using a set of eight peptidomimetic renin inhibitor analogs. Results from these studies provide suggestions for the design of compounds with improved bioavailability.

The bioavailability of a compound after oral administration is a function of molecular characteristics, dosage form design, and the barrier functions of the organism. Absolute bioavailability is defined as the dose-normalized area under the plasma concentration–time curve after oral administration divided by that after intravenous administration (Figure 1). This definition assumes that total body clearance of the compound is the same after different routes of dosing; hence, factors influencing bioavailability are those that relate to dosage form, intestinal absorption and metabolism, as well as first-pass of the compound through the liver[1] (Figure 2). A new chemical entity (NCE) is characterized by a set of physicochemical properties and the presence or absence of metabolically labile functionalities. Most of these characteristics can be measured or estimated early on in the discovery process, and can then be used to guide the selection of experiments designed to elucidate limits to the bioavailability of the compound. When tests have been established to assess these limitations, NCEs can be screened and structure–activity relationships (SARs) can be explored to optimize delivery and bioavailability.

The barriers to oral delivery of a compound can involve both stability and transport. After administration, the stability of an NCE is often challenged in the gastrointestinal (GI) tract. The GI tract encompasses a wide pH range, from approximately pH 1–2 in the stomach to above pH 7 in the lower small intestine. Residence time can be highly variable in the stomach owing to the various factors controlling gastric emptying. While the GI transit time is more consistent in the small intestine (3–4 h in humans), it is highly variable in the colon[2,3]. Hence, NCEs susceptible to acid- or base-catalyzed hydrolysis may be degraded before reaching sites of absorption. A host of hydrolytic and metabolic enzymes reside in the GI lumen, as well as in the cells lining the GI

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  1359-6446/96/$15.00. PII: S1359-6446(96)10039-8

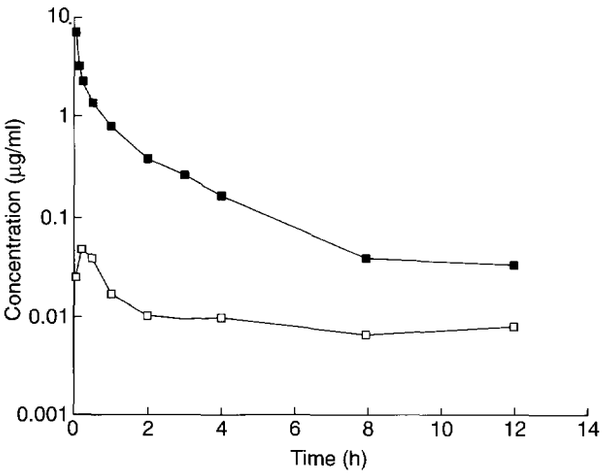

Figure 1. *Plasma concentrations of Cam 2445 in male Wistar rats after administration of a single intravenous (■, 5.2 mg/kg) or oral (□, 20 mg/kg) dose.*

tract. Given that many of these enzymes have evolved to digest proteins to a smaller size for absorption, NCEs of a peptidic nature may be particularly vulnerable.

In addition to stability limitations, poor absorption from the GI tract into the bloodstream may provide an additional barrier to high oral bioavailability[1] (Figure 2). Most compounds are absorbed by passive diffusion across the intestinal membrane or via the paracellular route, while others are substrates for carrier-mediated transport (Figure 3). Larger molecules may be absorbed by receptor-mediated endocytosis (transcytosis)[4]. One transport route generally dominates, but parallel pathways are not uncommon. Polarized efflux pumps located at the apical membrane may act counter to absorption and result in drug secretion[5,6]. For smaller molecules, the rate and extent of absorption by passive means will be largely dependent on molecular characteristics, such as aqueous solubility and lipophilicity. Permeation by the paracellular route is typically dictated by molecular size or radius[7]. The likelihood of absorption via a nutrient transporter can often be surmised by structural resemblance of the NCE to carbohydrates, amino acids or peptides, although several drug classes have also been described as substrates[8,9]. Complexation of the drug with metal ions in the GI tract may result in incomplete absorption[10,11].

Before reaching the systemic circulation, the absorbed NCE must pass through the liver, a major clearance and detoxification organ that is rich in metabolic enzymes[1] (Figure 2). In the liver, compounds may be metabolized to inactive or other active forms. Intact compound and metabolite(s) may be excreted, via the bile, back into the intestine, where reabsorption may occur. Susceptibility to biotransformation is largely determined by specific moieties, such as hydroxyl or methoxy groups, that are substrates for functional (Phase I) or conjugative (Phase II) metabolism. The likelihood of excretion into the bile appears to depend on a combination of physicochemical characteristics, including molecular size and lipophilicity[12].

Renin inhibitors (RIs) were extensively investigated in the pharmaceutical industry over the past decade, with the hope of obtaining more specific inhibitors of the renin–angiotensin system than the angiotensin-converting enzyme inhibitors used in the treatment of hypertension. Active RIs were designed using a number of synthetic strategies[13–15]. Structures were typically lipophilic, poorly soluble in water, of high molecular weight, and frequently of peptidic origin. Often subsequent to selection of a potent lead, studies in animals demonstrated unacceptably low bioavailability for RIs in a number of laboratories. As a result, systematic

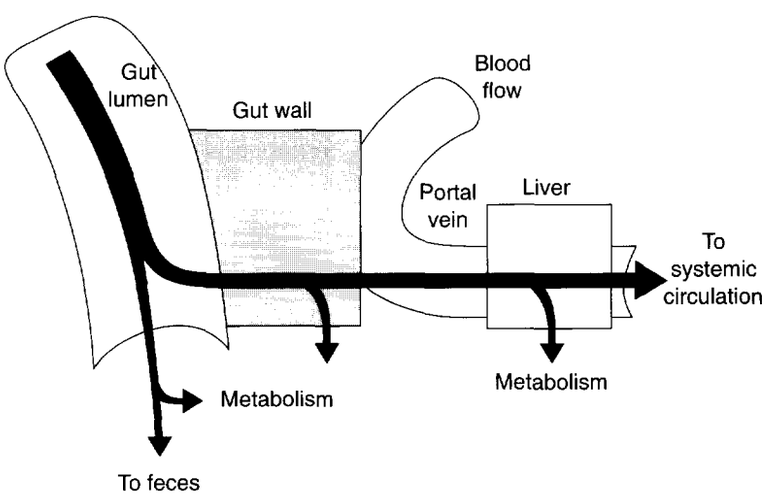

Figure 2. *An orally administered compound encounters several barriers and sites of loss in its sequential movement during gastrointestinal absorption. These limitations include incomplete dissolution, metabolism in the gut lumen or in the gut wall, and first-pass extraction in the liver. Reproduced with permission from Ref. 1.*

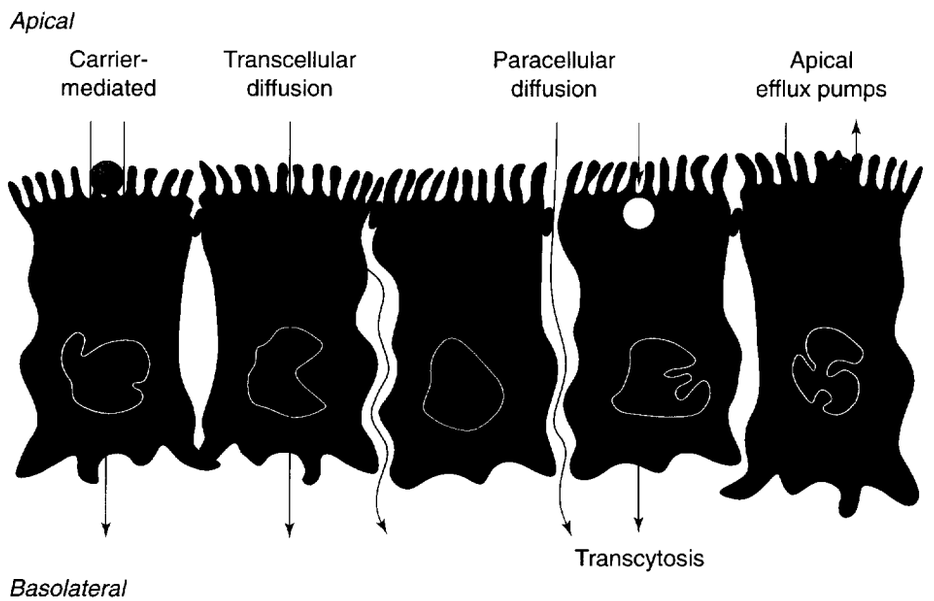

Figure 3. *Potential pathways for drug transport across a polarized epithelial cell monolayer. Pathways include carrier-mediated, transcellular and paracellular diffusion, as well as an efflux pump located in the apical membrane, and receptor-mediated endocytosis (transcytosis).*

studies were launched to increase understanding of the determinants of RI oral bioavailability, and experimental models were developed to isolate the physiological barriers of interest.

In this review, a series of eight RI analogs has been selected as a case study to illustrate the role of each of the above factors in determining oral bioavailability[16,17]. Not all compounds in the series possess RI activity. All analogs have the 2-amino-1-cyclohexyl-6-methylheptane-3,4-diol fragment (**1**, Figure 4) in common, which was tritiated at each position on the cyclohexyl ring for ready detection in biological fluids. **1** is the P1-P1′ isostere of the biologically active RI in this series, **4**. The bioavailability of **4** was less than 2% in rats. Analogs were selected for the study so as to comprise a wide range of physical properties, namely molecular weight, partition coefficient (*P*) and H-bonding.

Physicochemical properties

The physicochemical properties of an NCE will substantially affect its bioavailability after oral administration. From an early stage in drug discovery, it is advantageous to estimate several parameters to gain insight into the ease or difficulty of delivering the drug to its site of action. Calculation of molecular weight is straightforward after isolation and identification. Conventional wisdom holds that smaller molecules will be less problematic drugs. In particular, molecular size will impact on transport through biological membranes, with a relationship that is inversely proportional to the transport rate[18]. The RI analogs ranged in molecular weight from 243 to 809 (Table 1).

Aqueous solubility will dictate the amount of drug in solution and, hence, the amount available for absorption from the GI tract. Solid dosage forms containing compounds with low aqueous solubility may suffer from dissolution-rate-limited absorption. Incomplete absorption may result if the NCE does not completely dissolve within the GI residence time. Compounds administered in solution may precipitate in the GI tract, resulting in flip–flop pharmacokinetics when absorption is slower than elimination, and lowered bioavailability[19]. Low aqueous solubility is generally the result of lipophilic substituents in the NCE or of high intramolecular crystal lattice binding energy[20]. While the former is characterized by the partition coefficient (see below), the latter will be reflected by the melting point. Larger molecules may demonstrate complex solubility behavior because of the greater possibility of amphipathy. In the RI series, three of the analogs (**2**, **5** and **7**) were nonionizable, and therefore had limited potential for increases in aqueous solubility owing to changes in pH. The other five analogs had pK_as ranging from 4.7 to 9.3, and were zwitterionic (**8**), negatively (**6**) or positively (**1**, **3** and **4**) charged at physiological pH. For this series, analogs ranged from freely soluble (mg/ml) to poorly soluble (<5 μg/ml).

Lipophilicity and aqueous solubility are interrelated and often share a compensatory relationship: highly lipophilic compounds will typically have low aqueous solubility, and vice versa. Compounds with a balance between these two properties present the best case for achieving high oral

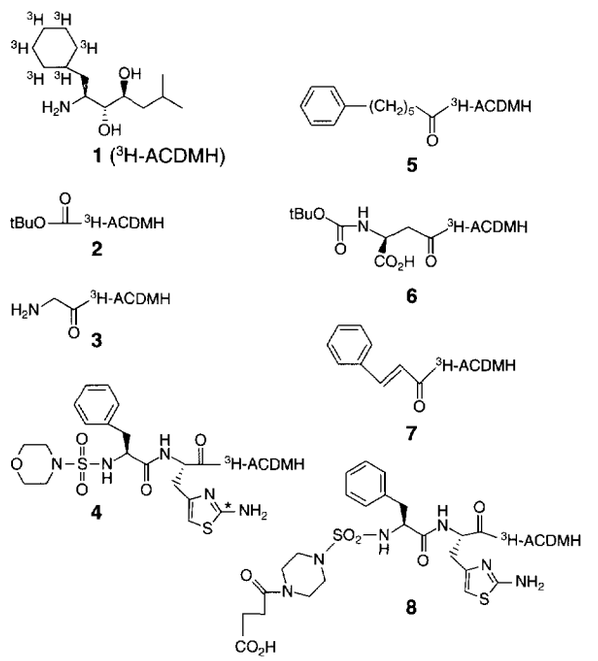

Figure 4. *Tritiated renin inhibitor analogs. **1** is 2-amino-1-cyclohexyl-6-methylheptane-3,4-diol (ACDMH), which was tritiated at each position in the cyclohexyl ring. For the bile duct cannulated rat studies, **4** was labeled with ^{14}C; the site of the ^{14}C label is indicated by an asterisk.*

bioavailability. Lipophilicity is a complex parameter, which can be estimated by calculation[21–24] or determined experimentally by several approaches. Traditionally, a shake-flask method has been used to assess partitioning between organic and aqueous phases[21]; however, for very lipophilic compounds (log $P > 4$), it may be difficult to analyze trace quantities of analyte in the aqueous phase. Correlation methods using C_{18} (Ref. 25) and immobilized artificial membrane[26] columns for HPLC have been developed for more rapid screening of NCEs. These techniques are based on different components of solute transfer across biological membranes, including desolvation and membrane partitioning. Useful first approximations of lipophilicity and membrane interaction can be obtained. The log P of this series of RI analogs were determined by an HPLC correlation method using an aqueous mobile phase at pH 6.5. Values were moderately to highly lipophilic, ranging from 1.5 to 4.7 (Table 1).

For compounds containing highly polar groups, such as some peptidomimetics, desolvation and the breaking of H-bonds may be the rate-limiting step to transfer across the membrane[27,28]. For such compounds, H-bonding capacity [calculated by summation of the N values (Table 2) assigned to the molecule's functional groups] may be a useful parameter[18]. The calculated value provides a good initial estimate, which may differ from the actual value if the molecule forms internal H-bonds. A nonaqueous partitioning system has been developed that yielded hydrophobicity values sensitive to changes in H-bonding capacity between solutes[29]. This hydrophobicity parameter correlated well with transport across Caco-2 cell monolayers for a series of peptides. H-bonding capacity was calculated for the RI analog series using the values listed in Table 2. The number of sites capable of forming H-bonds was very high, spanning 6–17.5 sites per molecule (Table 1). This unusually high number is characteristic of peptides and peptidomimetics, making them particularly difficult to optimize for pharmacokinetics.

Stability in the GI tract

Peptidic or peptidomimetic RIs often demonstrate low oral bioavailability because of instability in the GI tract. Acid-labile compounds can undergo chemical hydrolysis in the acidic environment in the stomach. In addition, the compounds can be challenged by nonspecific hydrolysis in the intestine, or by a host of metabolic enzymes in the GI tract. These enzymes include pepsin in the stomach, the pancreatic enzymes and various peptidases at the brush border region of the apical intestinal membrane and in the cytoplasm of the intestinal enterocytes[30]. The site of secretion and typical substrate of selected peptidases are listed in Table 3 (Refs 30–32). In addition to peptidases, other enzymes associated with the intestinal brush border membrane (BBM) include the carbohydrases, hydrolases and alkaline

Table 1. Selected physical properties of the renin inhibitor analog series

Compound	Log *P*[a]	Molecular weight	No. of H-bonds (*N*)[b]
1	1.9	243.4	6
2	4.0	343.5	6.5
3	1.5	300.4	8
4	3.0	708.0	13.5
5	4.7	417.6	6
6	2.1	458.6	11.5
7	3.6	373.5	6
8	2.0	808.0	17.5

[a]Log partition coefficient (*P*), determined at pH 6.5 by the HPLC correlation method.
[b]Number of sites capable of forming H-bonds (adapted from Ref. 18).

Table 2. Assignment of *N* values to H-bonding functional groups

Functionality	Examples	Assigned *N* value
–OH	Alcohols, sugars, glycols	2
	Carboxylic acids	2
H–O–H	Water	4
$–NH_2$	Primary amines	2
	Primary amides	2
–N(R)H	Secondary amines	1
	Secondary amides	1
–CO–	Carboxylic acids	1
	Amides	1
	Aldehydes	1
	Esters	0.5
–O–	Ethers	0
–C≡N	Nitriles	1
	Dicyandiamide	1

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phosphatases[33]. The amount of metabolic enzymes tends to decrease down the GI tract[34–36], while the amount of intestinal microbial flora increases. Large populations of microbial flora, found below the ileocecal valve and in the large intestine, are capable of both Phase I and Phase II metabolism[36–38]. Enzymes responsible for intracellular gut wall metabolism are found primarily in the villus tip cells rather than in the mucosal crypt cells. As the epithelial cells mature and migrate from the crypts to the mucosal villus tip, intestinal enzymes, including cytochrome P450, are synthesized[39,40]. CYP3A4 is the major isozyme present in the intestinal mucosa, although other liver cytochrome P450 enzymes are also found in the intestine[41–43]. Metabolic enzymes are localized in the smooth endoplasmic reticulum and, to a lesser extent, in mitochondria and cytoplasm[34,35]. These enzymes include the nonspecific esterases, phosphatases, dehydrogenases, oxidases and glucuronidases[33,35,36,39].

To examine the stability of the RI analogs in the GI tract, the compounds were incubated in buffer and rat intestinal perfusate, which was generated on the day of the experiment[16]. The perfusate[19] contained GI secretions, sloughed enterocytes and their contents, and enzymes bound to the mucosal surface. Compounds **1** and **4** were also incubated in pancreatic trypsin–chymotrypsin preparations and in rat jejunal BBM suspensions. The BBM suspensions, prepared as described by Tsuji and coworkers[8], were rich in membrane-bound enzymes from the intestinal brush border region. The samples were analyzed by HPLC and the fraction of drug remaining was monitored. All the RI analogs were stable in buffer and rat intestinal perfusate except compound **3**, which had a half-life of 3 h in the perfusate.

Table 3. Typical substrates of intestinal peptidases

Class	Peptidases	Site of secretion	Substrate[a]
Endopeptidase	Pepsin	Stomach	—●↓○— Phe, Trp, Tyr, Met, Leu
Endopeptidase	Trypsin	Pancreas	—●↓○— Lys, Arg
Endopeptidase	Chymotrypsin	Pancreas	—●↓○— Phe, Trp, Tyr
Endopeptidase	Endopeptidase 24.11 (EC 3.4.24.11)	Brush border	—○—○↓●—○—○— hydrophobic
Endopeptidase	Endopeptidase-2		—○—○↓●↓○—○— aromatic
Aminopeptidase	Aminopeptidase N (EC 3.4.11.2)	Brush border	●↓○—○—○— nonspecific
Aminopeptidase	Aminopeptidase A (EC 3.4.11.7)	Brush border	●↓○—○—○— Glu/Asp
Aminopeptidase	Aminopeptidase P (EC 3.4.11.9)	Brush border	○↓●—○—○ Pro
Aminopeptidase	Aminopeptidase W (EC 3.4.11.16)		○↓●—○—○ Trp
Carboxypeptidase	Carboxypeptidase P (EC 3.4.17)	Brush border	—○—○—●↓○ Pro, Ala, Gly
Dipeptidyl peptidase	Dipeptidyl peptidase IV (EC 3.4.14.5)	Brush border	○—●↓○—○ Pro, Ala
Peptidyl dipeptidase, endopeptidase	Angiotensin-converting enzyme (EC 3.4.15.1)	Brush border	—○—○↓●—● nonspecific
Dipeptidases	Dipeptidase (EC 3.4.13.2)	Cytosol	●↓● Gly-Leu
Omega peptidases	γ-Glutamyltranspeptidase (EC 2.3.2.2)	Brush border	●↓○—○—○— γ-Glu
Aminopeptidase	Aminotripeptidase	Cytosol	●↓○—○ Pro
Dipeptidase	Prolidase	Cytosol	○↓● Pro
Dipeptidase	Prolinase	Cytosol	●↓○ Pro
Dipeptidase	Carnosinase	Cytosol	●↓● β-Ala-His

[a]The arrows represent sites of cleavage of the peptidic bonds; solid circles indicate the location(s) of the specific amino acid(s) in the peptide substrates.

Both compounds **1** and **4** were stable in BBM. Compound **1** was also stable in preparations containing trypsin and chymotrypsin. The half-life of **4** in these preparations depended on enzyme concentration and ranged from 12 minutes to 4 h (Figure 5).

The results indicated that most of the RI analogs were stable in an environment approximating the GI lumen. Both the perfusate and BBM were enriched in GI enzymes, so that stability *in vivo* could be extrapolated if the compounds remained intact in these incubations. Compounds **4** and **8** both contain a hydrophobic Phe group, which makes the peptide bond involving the Phe carbonyl susceptible to chymotrypsin hydrolysis. The physiological concentration of chymotrypsin in the upper GI tract is reportedly 1.5–5.5 μM (Refs 44,45), suggesting that **4** would have a half-life of 1–4 h. Increases in GI transit time may therefore compromise the stability of compounds **3**, **4** and **8**.

Absorption and permeability

Peptidic or peptidomimetic RIs often suffer from low oral bioavailability because of limited permeability across the mucosal cells. Correlation curves have been established in several laboratories between intestinal permeability and fraction of dose absorbed[17,46–48]. In general, higher permeability is associated with the absorption of a higher fraction of the dose, until the curve plateaus at high permeability: 5×10^{-4} cm/min in the Caco-2 model or 20×10^{-4} cm/min in the rat intestinal perfusion model (Figure 6)[17]. For compounds absorbed by passive diffusion, an increase in lipophilicity is paralleled by an increase in membrane permeability, except for very lipophilic compounds (log $P > 4$); their permeability is limited by the aqueous boundary layer. Permeability experiments in rat intestinal perfusions or in Caco-2 cells have demonstrated that a sigmoidal relationship occurs between log P and effective permeability[48–52].

Increased intestinal flow or mixing can cause a reduction in the thickness of the aqueous boundary layer and an increase in effective permeability[49,51,53]. Recent reports in humans have indicated that the unstirred water layer is an insignificant barrier to intestinal absorption, probably owing to efficient lumenal mixing in the conscious subjects[54,55]. The absorption of highly permeable compounds, both by passive and active transport, is controlled by the cellular membrane rather than by the aqueous boundary layer[54,56]. In theory, the effect of the unstirred water layer on the rate and extent of absorption of highly permeable compounds in humans and animals is minimal[57].

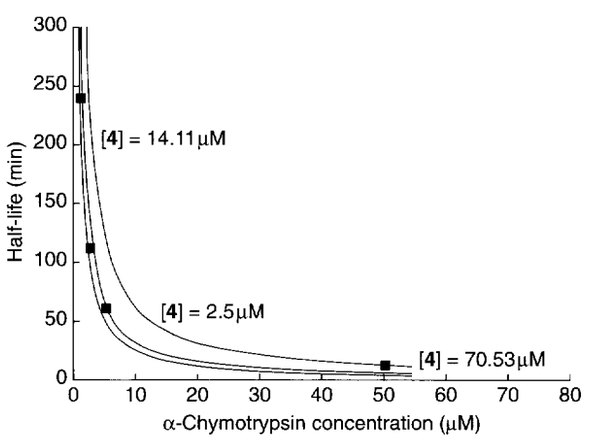

Figure 5. *Apparent half-life of* ***4*** *in preparations containing pancreatic enzymes (see text). Apparent half-life values were: 12 min at 50 μM each of α-chymotrypsin (CT) and trypsin (T); 61 min at 5 μM each of CT and T; 112 min at 2.5 μM each of CT and T; 240 min at 1 μM CT.* ■*, actual data; —, simulated data.*

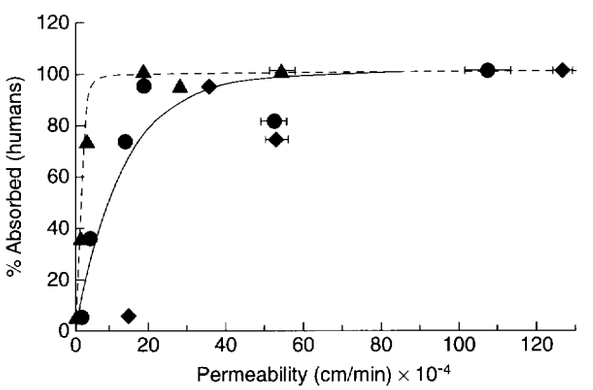

Figure 6. *Fraction of dose absorbed in humans versus permeability determined using rat everted intestinal rings (♦), rat intestinal perfusion (●), or Caco-2 cell monolayers (▲). The theoretical curves were generated using the equation* $F_{absorbed} = 1 - \exp(-2^{o}Peff^{*})$*, where* $^{o}Peff^{*}$ *is the dimensionless permeability. The curves were calculated by redimensionalizing* $^{o}Peff^{*}$ *using the ratio (D/r), with an average diffusitivity,* $D = 5\times10^{-4}$ cm^2/min*, and radius, r = 0.2 cm for rat intestine (—) or r = 2.0 cm for human intestine (---). Reproduced with permission from Ref. 17.*

Transcellular transport can also be affected by H-bonding potential. An increase in the number of H-bond-donor and -acceptor groups present is associated with a decrease in permeability and percentage of dose absorbed[58–60]. Transport of hydrophilic compounds, including ionized species, peptides and nucleosides, is restricted across the cell because of the lipoidal nature of the membrane, but can be mediated by specialized carriers or by the paracellular pathway[61–63]. Paracellular transport is essentially limited to compounds of low molecular weight (<400–500 in Caco-2 cell monolayers, rat ileum and colon)[64]. Higher molecular weight (MW) is generally associated with lower permeability because the rate of diffusion of a drug in water is inversely proportional to $MW^{1/3}$ (Ref. 18). The ability of a molecule to traverse the paracellular pathway is dependent on molecular size, shape, charge and other physicochemical properties, as well as on the dynamic width and selectivity of the tight junctions[7,61,64,65]. The epithelium becomes tighter and the size restriction more stringent as a function of distance down the intestinal tract[48]. Solvent drag in the intestine induced by hypotonic solution or sodium-coupled nutrient transport results in increased paracellular transport of hydrophilic compounds in animal perfusions and in Caco-2 cells; however, similar results have not been observed at physiological lumenal flow in humans[65]. Absorption of compounds via the paracellular pathway, particularly those with a molecular weight above 200, will probably be low in humans[65]. Ionized species of lower molecular weight may utilize the paracellular pathway. Alternatively, lipid-soluble prodrugs may be synthesized to mask the charges and increase lipophilicity for transcellular transport (e.g. ester prodrugs for carboxylic acid compounds)[66,67]. Although interaction of RIs with the intestinal peptide transporter has been reported[68], the compounds in this present RI analog series were probably transported by passive diffusion. Carrier-mediated transport is unlikely to occur because of the chemical structure of the RI analogs.

The intestinal permeabilities of the RI analogs were examined in three different models: *in situ* rat intestinal perfusions, *in vitro* rat intestinal ring uptake, and Caco-2 cell monolayers from human colon adenocarcinoma[17]. Each of these models examined absorption of the drugs from solution, thus removing the possibility of rate limitation through dissolution. The intestinal perfusion model involved single-pass perfusion of the drug solution through a selected intestinal segment. Permeability was calculated from the steady-state disappearance of the drug from the intestinal lumen. The rat intestinal ring method entailed incubation of everted intestinal rings in the drug solutions. Ring permeability was calculated by measuring accumulation of the compound in the tissue under initial rate conditions. The Caco-2 cell monolayers were used in a side-by-side diffusion apparatus, wherein the drug solution was placed in the donor compartment and permeability was based on the rate of appearance of compound in the receiver compartment.

The permeabilities of the RI analogs obtained in the three experimental systems are tabulated in Table 4. There was good agreement between the different models in ranking the permeabilities. The trend in permeability rank order for the RI analogs in the three systems was rings > perfusion ≥ Caco-2, which was similar to the rank order of the reference compounds.

The relationships between the physicochemical parameters and permeabilities of the RI analogs derived from the rat intestinal perfusion experiments are depicted in Figure 7. In general, permeabilities increased with lipophilicity (Figure 7a). The RI analogs were moderately to highly lipophilic, with partition coefficients spanning three orders of magnitude. The neutral compounds, being least hydrophilic, exhibited high permeability, suggesting complete absorption from the intestine (Figure 6 and Table 4). The ionized compounds demonstrated low to moderate permeability, with the cationic compounds more permeable than the

Table 4. Permeability of peptidomimetics: everted intestinal ring, Caco-2 and intestinal perfusion permeabilities[a]

Compound	Permeability (cm/min) × 10^{-4}		
	Ring[b]	Caco-2[c]	Perfusion[d]
1	109±14.0	14.8±0.9	27.1±2.5
2	316±98.3	40.0±6.7	88.5±9.5
3	19.9±6.8	16.1±1.2	12.1±1.6
4	15.9±6.5	3.4±0.8	21.6±3.19
5	54.2±21.0	4.2±0.1	90.8±10.3
6	36.7±21.1	5.4±0.5	5.9±0.9
7	212±109	51.2±8.6	82.0±8.2
8	ND[e]	3.9±0.2	2.6±0.7

[a]Values for perfusion experiments represent the mean of 4–8 animals; for Caco-2 experiments, the mean of 3–6 cell monolayers; for ring experiments, the extrapolated value from three time courses, where each time course consisted of 21–27 rings from one animal. Standard errors are tabulated with the mean values.
[b]*In vitro* rat everted intestinal ring uptake.
[c]Caco-2 cell monolayers from human colon adenocarcinoma.
[d]*In situ* single-pass rat intestinal perfusions.
[e]ND, not determined.

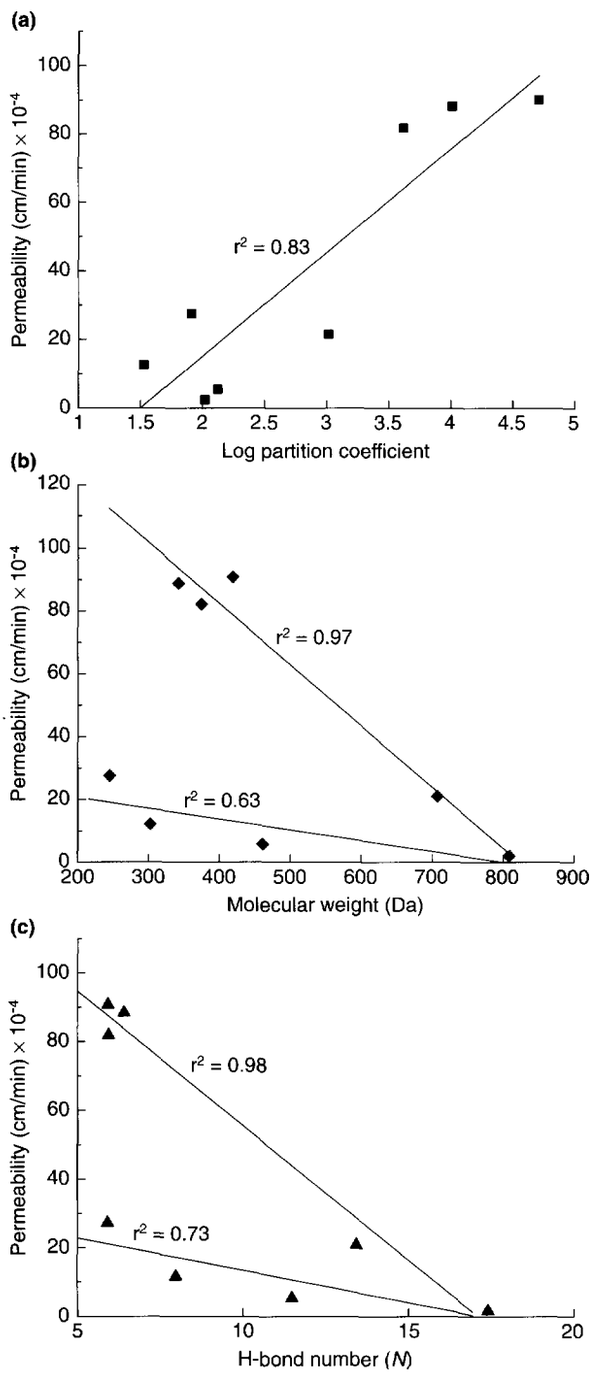

Figure 7. *Permeabilities of renin inhibitor analogs in rat intestinal perfusion experiments as a function of: (a) lipophilicity, expressed as log partition coefficient (P); (b) molecular weight (upper curve: neutral compounds and compound* ***4****; lower curve: ionized compounds); (c) H-bonding potential, expressed as H-bond number (N) (upper curve: neutral compounds and compound* ***4****; lower curve: ionized compounds).*

anionic or amphiphilic compounds. The absorption of the more lipophilic compounds may be limited by the aqueous boundary layer, but the plot did not definitively plateau at higher partition coefficients. Permeabilities of the RI analogs decreased with increasing molecular weight (Figure 7b) or increasing H-bonding potential (Figure 7c). However, molecular weight was highly correlated with the number of H-bonds possible for the RI analogs, and individual contributions could not be delineated. The compounds appeared to fall into two categories, one comprising the charged compounds, the other group including the neutral compounds, as well as the RI with the lowest fraction ionized at physiological pH (**4**). Within each group, compounds with larger molecular size or involving higher solvation energy exhibited lower permeabilities.

Hepatic first-pass

Compounds absorbed into the portal blood can be efficiently extracted by the liver, particularly during first-pass after absorption. Of the cytochrome P450 superfamily, CYP3A enzymes represent the most abundant isoforms in the liver and they are responsible for the metabolism of xenobiotics of diverse chemical structure[69–71]. The uptake of a drug into hepatocytes may be mediated by passive or carrier processes[72,73]. Once in the parenchymal cell of the liver, the drug may be metabolized or it could bind to intracellular proteins (Figure 8). The drug or its metabolite(s) may return to the circulation or exit from the hepatocyte into the bile canaliculus, again by passive or carrier-mediated transport[73–75], before secretion in bile[12]. Experimental systems have been devised to study these processes in isolation, for example uptake by hepatocytes[76] or bile canalicular membrane vesicles[77], and in partial or full complexity, for example isolated perfused rat liver (IPRL)[78] and bile duct cannulated (BDC) rat models.

First-pass extraction is governed by transport at multiple barriers, as well as by potential binding and metabolic processes. Designing NCEs that avoid or minimize extensive first-pass extraction is difficult because of the high degree of coupling between transport, binding and metabolism. Compounds with small differences in physical properties or substituents may exhibit large differences in hepatic processing. Molecular weight and hydrophobicity appear to correlate with first-pass extraction and secretion in bile. In the IPRL model, the uptake of a set of tetrapeptides was found to correlate with hydrophobicity (Figure 9)[79]. In the intact animal, recovery of a series of poly(ethylene glycol)

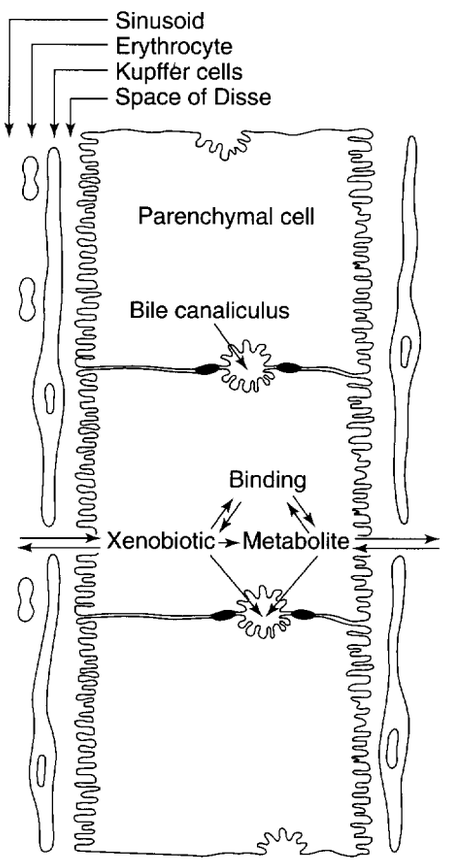

Figure 8. *Contact of substances in plasma with cells of the liver. Reproduced with permission from Ref. 12.*

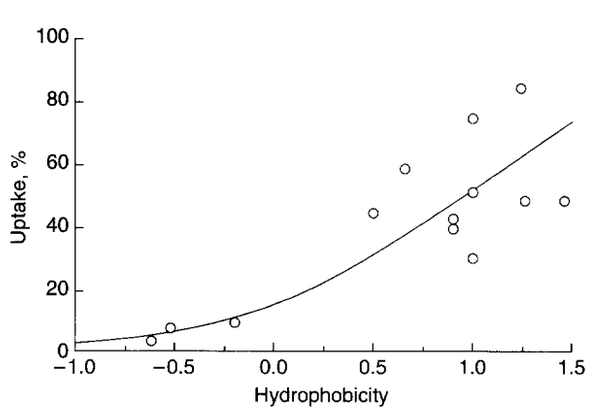

Figure 9. *First-pass extraction as a function of hydrophobicity for tetrapeptides as measured in isolated perfused rat liver studies. Reproduced with permission from Ref. 79.*

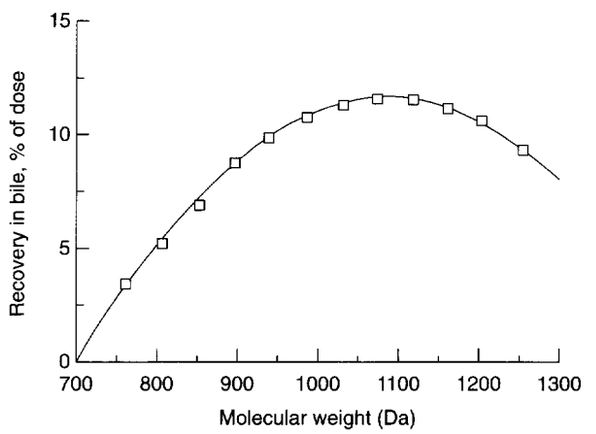

Figure 10. *Correlation between the molecular weight of poly(ethylene glycol) polymers and the percentage of the dose recovered in cat bile. Reproduced with permission from Ref. 80.*

polymers in cat bile increased with molecular weight, up to a maximum of approximately 1100 (Figure 10)[80]. In general, the more hydrophobic the compound, the greater the extent of hepatic extraction. The higher the molecular weight, the greater the extent of hepatic extraction, up to a maximum molecular weight, which is dependent on both the compound and the species of animal being studied.

IPRL studies

Hepatic extraction of the RI analogs was examined in an IPRL model[16]. Selected ^{3}H-labeled compounds were perfused in the preparation using the recirculating model (Figure 11). Drugs were added to the reservoir as a bolus. The perfusate was sampled at selected time points and bile was collected. The amount of drug in the liver was determined by mass balance. In the 2-h recirculating liver perfusions, the compounds disappeared rapidly from the perfusate, although fates differed after the initial uptake. As shown in Figure 12a, the radioactivity from **1** effluxed back into the perfusate within 30 min, whereas the major portion of the radioactivity from **4** was secreted into the bile (Figure 12b). The radioactivity distribution of the other RI analogs was intermediate to **1** and **4** (Figure 13). The maximum bile elimination rate was also found to correlate with molecular weight (Figure 14a) and probably with lipophilicity, with differentiation between ionized and uncharged compounds (Figure 14b).

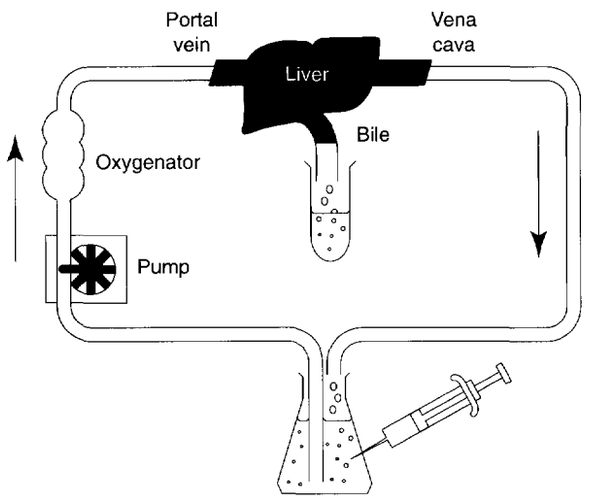

Figure 11. *Experimental set-up for recirculating models of hepatic extraction in isolated perfused rat liver. Reproduced with permission from Ref. 16.*

BDC rat studies

Biliary clearance is a major excretion pathway for compounds of molecular weight 400–850, particularly those that are amphipathic[12], and has been implicated in the short duration of action observed after intravenous administration of RIs (Refs 15,81). The BDC rat model permits evaluation of degradation in the GI tract, extent of absorption and biliary excretion, and can thus be a useful *in vivo* model to elucidate limits to bioavailability. ^{3}H-labeled **1** or ^{14}C-labeled **4** was administered orally or intravenously to surgically prepared animals[82]. Urine, bile and feces were collected at selected time points, with replacement of bile supplied by control animals. The GI tract and its contents, as well as the carcass, were harvested at the end of the experiment. Samples were analyzed by total radioactivity (TRA), as well as by radioflow detection after HPLC separation. The intestinal absorption of **1** (Table 5), the P1-P1′ isostere of **4**, was approximately 2.5-fold higher than that of **4** (Table 6). This result may be due to the larger molecular weight of **4** (709 versus 243) and slower rate of diffusion. Because of its lower aqueous solubility, **4** may have precipitated in the GI tract, further limiting the extent of its absorption. Twenty-four hours after oral administration, less than 20% of either compound remained intact in the GI tract. Despite a low mass balance recovery for **1**, it was clear from TRA analysis that this compound was preferentially excreted via the renal pathway, while **4** appeared largely in the bile. This finding

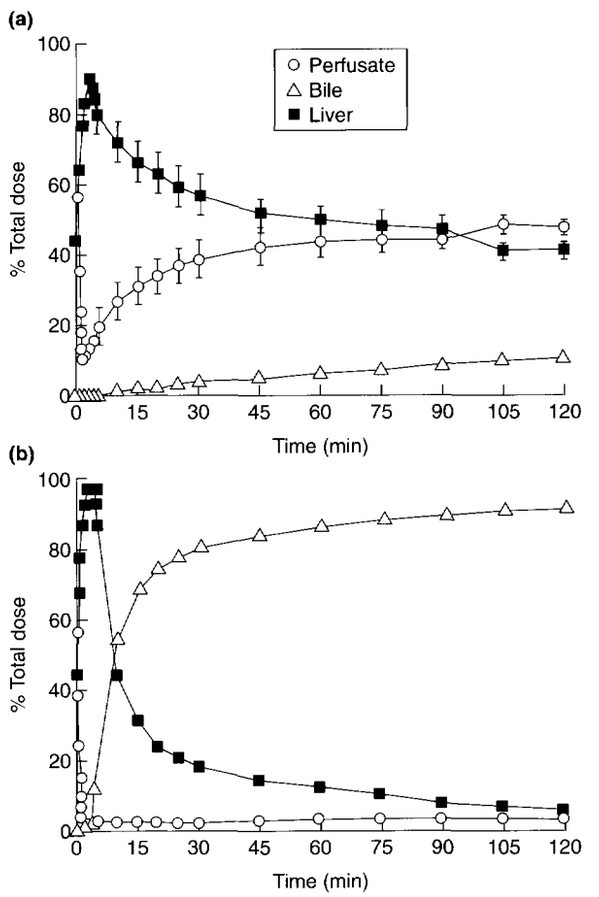

Figure 12. *Fate of (a) **1** and (b) **4** in recirculating isolated perfused rat liver. Reproduced with permission from Ref. 16.*

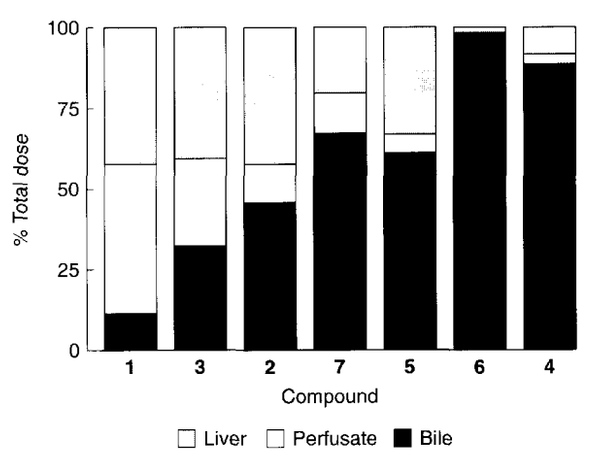

Figure 13. *Distribution of renin inhibitor analog radioactivity between perfusate, bile and liver. Reproduced with permission from Ref. 16.*

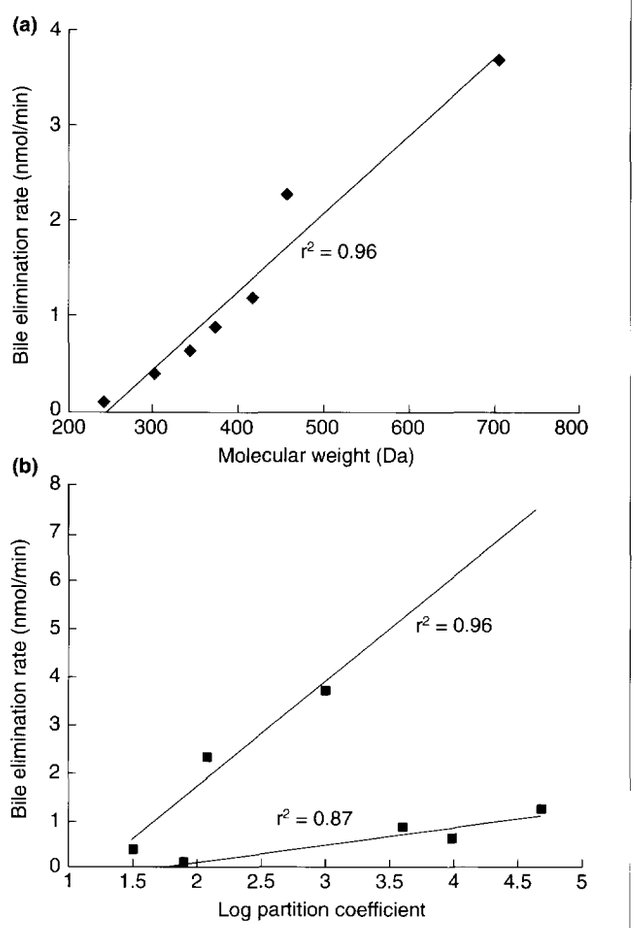

Figure 14. *Biliary clearance of renin inhibitor analogs from isolated perfused rat liver as a function of: (a) molecular weight; (b) lipophilicity, expressed as log partition coefficient (P) (upper curve: ionized compounds; lower curve: neutral compounds). Adapted from Ref. 16.*

was attributed to the larger molecular weight and higher lipophilicity of **4**. Results were thus consistent with those in the recirculating IPRL model described above. Both compounds were extensively metabolized. Approximately 5% of **4** was detected as intact drug in the bile and urine after intravenous administration. No intact **1** was detected in bile or urine after intravenous administration. Given the rapid clearance and extensive metabolism of **4** after intravenous administration (Figure 15), it is likely that the low bioavailability of **4** resulted from poor absorption after oral administration.

Conclusions

Bioavailability is a function of intestinal absorption and first-pass elimination. The extent of intestinal absorption is dependent on the stability of a drug in the GI tract, its aqueous solubility, and its permeability through the intestinal membrane. First-pass elimination may be effected by intestinal-mucosal or hepatic processing. Results with a set of RI analogs showed that most of the compounds were stable under *in vitro* conditions simulating those in the GI tract. Our results support those in the literature, indicating that intestinal permeability increases with lipophilicity, but decreases with molecular weight or H-bonding potential. There was rapid hepatic uptake of all RI analogs in the recirculating IPRL model, although the fates of the NCEs differed after initial uptake. Our results corroborate those in the literature in that elimination in the bile correlates with molecular weight and, to a lesser extent, with lipophilicity for the RI analogs. NCEs with the best potential for high oral bioavailability balance a combination of physicochemical characteristics, including lower molecular weight, reasonable solubility in water, moderate lipophilicity and low H-bonding capacity.

Table 5. Total recovery of radioactivity (TRA) and of intact compound 1 after intravenous or oral administration of ^{3}H-labeled compound 1 to bile duct cannulated rats[a]

	Intravenous administration		Oral administration	
	TRA (%)	Recovery of **1** (%)	TRA (%)	Recovery of **1** (%)
Urine	38.26±6.04	0	48.18±6.43	0
Bile	6.08±0.04	0	6.86±2.09	0
Gastrointestinal tract	1.20±0.06	0.04±0.02	10.35±6.33	7.67±6.89
Carcass	5.85±0.16	ND[b]	11.38±2.04	ND[b]
Total	51.39±6.09	0.04±0.02	76.78±2.90	7.69±6.89
Percent absorbed	–	–	66.42±6.64	–

[a]The intravenous dose was 0.26 mg/kg (115 μCi), $n = 3$; the oral dose was 0.53 mg/kg (130 μCi), $n = 4$. Mean values ± standard deviation are reported.

The fraction of dose absorbed was calculated as: dose (%) in [urine + bile + carcass].

[b]ND, not determined.

Table 6. Total recovery of radioactivity (TRA) and intact 4 after intravenous or oral adminstration of ^{14}C-labeled compound 4 to rats[a]

	Intravenous administration		Oral administration	
	TRA (%)	Recovery of **4** (%)	TRA (%)	Recovery of **4** (%)
Urine	8.24±1.74	2.94±0.92	3.40±1.59	0.06±0.11
Bile	78.20±4.90	2.09±0.81	17.96±13.14	0.57±0.59
Gastrointestinal tract	3.17±0.70	0	64.36±14.28	16.21±9.15
Carcass	5.66±2.56	ND[b]	3.77±0.59	ND[b]
Total	95.30±4.46	5.00±0.65	89.49±4.58	16.83±8.67
Percent absorbed	–	–	25.14±13.02	–

[a]The intravenous dose was 1 mg/kg (10 μCi), n = 4; the oral dose was 2 mg/kg (20 μCi), n = 4. Mean values with standard deviation are reported. The fraction of dose absorbed was calculated as: dose (%) in [urine + bile + carcass].
[b]ND, not determined.

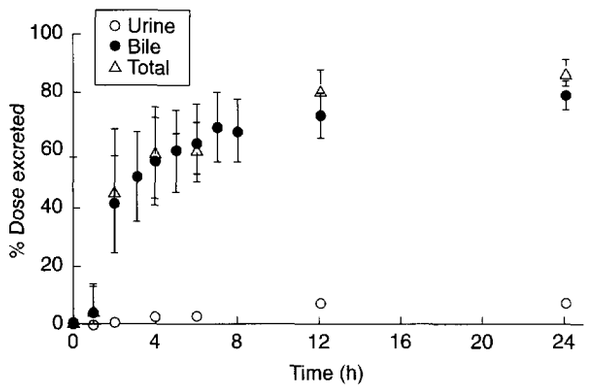

Figure 15. *Time course of recovery of ^{14}C activity following intravenous administration of ^{14}C-labeled **4** (1 mg/kg, 10 μCi) to bile duct cannulated rats* (n=4).

ACKNOWLEDGEMENTS

The authors thank Robert Bonczyk and James Burleigh for their animal support work in the original *in vivo* research discussed in this review. We also thank David Fleisher for his critical reading of the manuscript and Theresa Davis for secretarial assistance.

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In short ...

At the recent ACS meeting, **Silicon Graphics** and **Tripos** announced a joint project – dubbed SpaceCrunch – that will demonstrate 'how the union of high-performance scalable supercomputing and leading edge database technology can fundamentally change the drug discovery process.' The project, based at Silicon Graphics in Switzerland, is set to create a 100-billion-compound chemical database, the largest database of its kind, using Silicon Graphics' POWER CHALLENGEarray™ supercomputer and Tripos' ChemSpace™ technology.

A new drug research company was launched in the UK last month. **Pharmagene Laboratories**, based in Royston, specializes in the discovery of new medicines through the study of the expression and function of genes and gene products in human tissue. It is the first drug company to do research exclusively on human tissue; no animal experiments will be carried out. The company was founded by Dr G. Smith-Baxter (formerly SmithKline Beecham) and Dr R. Coleman (formerly GlaxoWellcome), together with a group of expert biochemists, molecular biologists and pharmacologists. Pharmagene is already working with several pharmaceutical partners to provide human pre-clinical data for their drug discovery programmes.

Roberts Pharmaceutical Corporation, an international pharmaceutical company that focuses on the acquisition and development of late-stage drugs, has received FDA clearance for the marketing in the US of their first pipeline drug, ProAmatine™. ProAmatine (midodrine hydrochloride) is a treatment for orthostatic hypotension, a low blood pressure condition.

Oxford BioMedica, a UK-based gene therapy company, has raised £750,000 with pre-placement of stock and now announces its intention to float. The company was set up earlier this year by Professor A. Kingsman and Dr S. Kingsman from Oxford University's internationally reputed Retrovirus Molecular Biology Group to commercialize new retrovirus technology for the delivery of therapeutic genes to human cells (gene therapy). Oxford BioMedica will retain a close relationship with Oxford University and is expected to commercialize further technologies developed in the University's laboratories.