Physiological models of hepatic extraction have been used quite successfully to predict in vivo clearance from in vitro data. The well-stirred model (Pang and Rowland, 1977) is most frequently used, owing in part to its simplicity. However, a similar application of the well-stirred model to the intestine was less successful (Thummel et al., 1985; Watkins, 1997). A possible reason for its failure might be the polarized expression of drug-metabolizing enzymes within the intestine (Chowdhury et al., 1985; Watkins, 1997). Most enzymes involved in intestinal elimination, such as the UDP-glucuronosyltransferases (UGTs), cytochrome P450 3A (CYP3A), and efflux pumps such as P-glycoprotein, are expressed in the epithelial cells (Chowdhury et al., 1985; Watkins, 1997). On the other hand, the vasculature (“serosa”) is devoid of significant enzymatic activity. This seems to refute the premise of the intestine as a well-stirred organ.

Because the intestine can receive compounds luminally and vascularly, elimination could occur both during and after absorption, phenomena called pre- and postabsorptive intestinal elimination (Routledge and Shand, 1979). The polarized intestinal tissue might allow drug administered perorally to have better access to these eliminating sites than that administered vascularily, leading to greater intestinal extraction and possible qualitative differences in metabolite kinetics, as well. Thus, intestinal clearance is potentially a function of intrinsic clearance as well as the ability of the drug to diffuse to the epithelial eliminating site, i.e., its diffusional clearance. An assessment of these factors led Gwilt and colleagues (1988) to model the intestine as three compartments: the lumen, the metabolically active mucosa, and the blood in equilibrium with the gut tissue, which can be considered the serosa (Gwilt et al., 1988). These authors proposed that differences in pre- and postabsorptive intestinal elimination could arise due to a “diffusional barrier” at the mucosa-serosa interface. A diffusional barrier is not necessarily a membranous barrier per se but could be an increased diffusional path length as a consequence of the polarized localization of intestinal enzymes.

Phenol, the model substrate used in the present study, is extensively metabolized by the rat gut (Cassidy and Houston, 1984). It has a simple biotransformational fate, being largely converted to a sulfate metabolite and a glucuronide with essentially all of the metabolites being renally eliminated (Capel et al., 1972). Phenol is glucuronidated by members of the UGT1A family (Inoue et al., 1999) and sulfated by the phenol sulfotransferases (Oddy et al., 1997). The UGTs show considerable intestinal expression in rats (Moijarrabi and Mackenzie, 1998; Inoue et al., 1999; Tukey and Strassburg, 2000). The UGTs display a decreasing expression gradient in epithelial cells from the villus to the crypt (Chowdhury et al., 1985) and are localized between the nuclear and apical membrane of the epithelial cell (Inoue et al., 1999). The active site of the UGTs is found within the endoplasmic reticulum (ER)
lungs (Burchell and Coughtrie, 1989). The phenol sulfotransferases (PSTs) are also found in the intestinal mucosa (Dunn and Klaasen, 1998) and are cytosolic enzymes (Burchell and Coughtrie, 1997). Thus, both enzymes display some form of tissue and cellular localization.

The objectives of the present study were to establish a relationship between intestinal organ clearance and its putative determinants, namely, diffusional clearance, intrinsic clearance, and intestinal blood flow, and to evaluate the role of enzyme localization on intestinal metabolism. Phenol was administered vascularity and luminally to the recirculating in situ perfused intestine (IPI) and the IPI with luminal dosing. The influence of tissue and cellular localization of drug-metabolizing enzymes on intestinal organ clearance and metabolite kinetics was studied.

Materials and Methods

Phenol, phenyl glucuronide, p-cresol, sodium pentobarbital, glucose, bovine serum albumin (25% in Tyrode’s solution), and sulfatase (type H-1 from Helix pomatia) were all obtained from Sigma-Aldrich (St. Louis, MO). Dextran T-40 was obtained from Amersham Biosciences (Piscataway, NJ). All other chemicals were reagent grade or better. Packed human red blood cells were obtained from the American Red Cross (St. Paul, MN).

Vascular Dosing: The Recirculating IPI. Male Sprague-Dawley rats, 250 to 275 g (Harlan, Indianapolis, IN), were used. Surgery was conducted with minor modification of a procedure described previously (Hirayama et al., 1989). The perfusate was Krebs-Henseleit bicarbonate buffer (pH 7.4), bovine serum albumin, Dextran T-40, and washed human red blood cells in approximate proportions (Hirayama et al., 1989), and the superior mesenteric arterial perfusate flow rate was 7.5 ml/min (Davies and Morris, 1993). The effluent perfusate from the portal vein was returned to the reservoir. A small incision was made in the jejunum to accommodate a glass cannula, and luminal fluid was collected during the course of the experiment. The viability of the perfused organs was continuously monitored by means of blood pressure at the superior mesenteric arterial perfusate.

Surgery was performed on anesthetized male Sprague-Dawley rats to initiate a blank IPI. The blank vascular perfusate was recirculated until the preparation stabilized at 37°C. The jejunum was ligated 2 to 3 cm from the ligament of Treitz, and a loose ligature was placed 15 cm distal to it. A small incision was made in the jejunum at this point, and a metal feeding tube was secured in it by tightening the ligature around it. The hub of the feeding tube was attached to a glass syringe containing the dose, and this was introduced as a bolus at time 0. Samples (300 µl) were taken from the reservoir and portal vein at 3, 8, 15, 30, 45, and 60 min. In addition, a reservoir sample taken before the start of the experiment was used to calculate the dose. Samples were centrifuged at 13,000 rpm for 2 min (Fisher Microcentrifuge, model 250B, Fisher Scientific, Pittsburgh, PA). The supernatant was stored at −20°C until further analysis.

Luminal Dosing: In Situ Perfused Intestine with Luminal Dosing. Surgery was performed on anesthetized male Sprague-Dawley rats to initiate a blank IPI. The blank vascular perfusate was recirculated until the preparation stabilized at 37°C. The jejunum was ligated 2 to 3 cm from the ligament of Treitz, and a loose ligature was placed 15 cm distal to it. A small incision was made in the jejunum at this point, and a metal feeding tube was secured in it by tightening the ligature around it. The hub of the feeding tube was attached to a glass syringe containing the dose, and this was introduced as a bolus at time 0. Samples (300 µl) were taken from the reservoir and portal vein at 0, 10, 25, 55, and 70 min and from the portal effluent at 2.5, 10, 30, 55, and 70 min. Sample handling was as described above. At the end of the experiment, the jejunum was flushed with air and saline. The luminal fluid collected was used to determine the amount in the lumen at the end of the experiment and, when subtracted from the original dose, gave the amount absorbed during the course of the experiment. Two luminal doses of phenol (100 and 1000 µg) were administered.

Analytical Methods. Phenol samples were analyzed by HPLC with fluorescence detection. The HPLC setup consisted of a Beckman Gold 126 system (Beckman Coulter, Inc., Fullerton, CA) with Gold Nouveau integration software (version 1.6; Beckman Coulter) and a RP 530 fluorescence detector (Shimadzu Corp., Kyoto, Japan). A reversed-phase C18 Supelcosil (4.6 × 250 mm; 5 µm particle size; Supelco, Bellefonte, PA) column was used. The mobile phase consisted of buffer and acetonitrile (75:25 parts by volume) delivered at a flow rate of 1 ml/min with isocratic elution. The buffer was 0.05 M KH2PO4 (Fisher Scientific) with 5 mM tetrabutylammonium phosphate (Regis Technologies, Inc., Morton Grove, IL) as an ion-pairing agent. The pH of the buffer was adjusted to 5.5 with 1% phosphoric acid. The excitation and emission wavelengths were 260 and 305 nm, respectively.

Standard curves of 1 to 80 µg/ml and 2.5 to 100 µg/ml were constructed for phenol and phenyl glucuronide, respectively. The internal standard was p-cresol (50 µg/ml). The retention times for phenyl glucuronide, phenol, and p-cresol were 3.9, 8.3, and 13.7 min, respectively. A phenyl sulfate standard was not commercially available; hence, an enzymatic incubation with sulfatase was used. An aliquot of 200 µl of the sample supernatant was split into two 100-µl portions. One part was assayed for phenol and the glucuronide as described above. The other 100 µl was incubated with 40 µl of sulfatase (5000 U/ml) for 16 h in a shaking water bath at 37°C. The difference between the phenol concentration before and after sulfatase incubation yielded the concentration of phenyl sulfate. Because a phenyl glucuronide standard was available, it could be shown that the sulfatase preparations were devoid of glucuronidase activity. There was no decrease in phenyl glucuronide concentrations in perfusate samples undergoing sulfatase incubation (data not shown).

The HPLC assay for phenol and phenyl glucuronide was validated for inter- and intraday precision (% coefficient of variation, % CV) and accuracy. These were found to be within acceptable limits, with a % CV and bias of less than 10%, except for the lowest concentration of the phenyl glucuronide, which had a % CV of approximately 15% (Kothare, 2001).

Data Analysis. The actual vascular dose administered was calculated by multiplying the initial concentration of drug in the reservoir by the volume of the reservoir. Due to the surgical setup, the gut was the only eliminating organ in the "circuit". Thus, the postabsorptive gut clearance, CLgw,org, could be calculated by

$$\text{CL}_{gw,org} = \frac{\text{Dose}}{\text{AUC}_{res}}$$

where AUCres is the area under the concentration-time curve of the drug in the reservoir from 0 to ∞ (Rowland et al., 1973). The AUCres from 0 to the last experimental time point, t, was calculated by the linear trapezoidal rule. The concentration-time profile was fit exponentially with KaleidaGraph (version 3.5; Abbebeck/Synergy, Reading, PA) to obtain a terminal slope, λ. The AUC from the last time point to infinity was calculated by dividing the concentration at the last time point by λ. The two partial AUCs were added to obtain the AUC from 0 to infinity. The intestinal extraction ratio, Egw, was calculated as

$$E_{gw} = \frac{\text{CL}_{gw,org}}{Q_{res}}$$

where Qres was the perfusate flow rate.

After luminal dosing, the preabsorptive clearance was calculated based on an equation developed from the model in Fig. 1. In the figure, Cgw, Cpw, and Cgw, in were the concentrations of the drug in the reservoir, the gut, and entering the gut wall, respectively. Cpw was the concentration of the drug leaving the gut wall. The rate of drug entry from the lumen to the gut wall was given by dX/dt. The preabsorptive clearance calculated was the intrinsic clearance (CLpw) of the gut. The concentration entering the gut wall, Cgw, in was considered equivalent to the concentration leaving the reservoir, Cgw. The concentration leaving the gut wall, Cgw, was assumed to be equivalent to the concentration within the gut wall, Cgw. This assumption is justified for phenol because it has a very high free fraction (approximately 86%) in a similar perfusion medium (Ballinger et al., 1995); hence, the free drug may be considered to be in a rapid equilibrium between the tissue water and the perfusate.

A mass balance equation for the rate of change of drug amount with time within the gut wall (dgw/dt) was obtained:

$$\frac{dgw}{dt} = \text{Rate in} - \text{Rate out}$$

$$= \frac{dX}{dt} + Q_{gw} \cdot (C_{gw, in} - CL_{gw} \cdot C_{gw} - Q_{gw} \cdot C_{pw})$$

Substituting the assumptions of the model in the above equation:
The mesenteric vessels appeared to collapse.

Vascular Dosing of Phenol with Varying Vascular Flow Rates. Experiments were conducted at flow rates of 5 (n = 3), 10 (n = 2), and 12 (n = 2) ml/min with 2500 μg vascular doses of phenol. In addition, data were used from the aforementioned study in vascularly dosed rats with a flow rate of 7.5 ml/min. Flow rates of less than 5 ml/min were attempted but were unsuccessful because the mesenteric vessels appeared to collapse.

Theoretical Evaluation of Intestinal Clearance. The data analyses described in the preceding section regarded the gut wall as a single compartment, and the pre- and postabsorptive intestinal clearances calculated were the intrinsic clearance and organ clearance by the gut, respectively. To get a clearer understanding of the physiological determinants of intestinal clearance, a more rigorous theoretical analysis was done; the model employed is shown in Fig. 2. The gut wall was divided into two compartments, the eliminating mucosa and the non-eliminating serosa. A diffusional clearance, $CL_{diff}$, governed mass transfer between the two compartments. The intrinsic clearance from the mucosa, $CL_{gw,org}$, was responsible for intestinal elimination. Both vascular and luminal dosing cases were evaluated. The detailed derivations are provided in Appendix I.

Based on this analysis, after vascular dosing of drug, the fraction escaping gut wall clearance, $f_g$, was given by:

$$f_g = \frac{Q \cdot (CL_{diff} + CL_{gw})}{CL_{diff} \cdot CL_{gw} + CL_{diff} \cdot Q + CL_{gw} \cdot Q}$$

where

- $Q$ = intestinal blood flow
- $CL_{diff}$ = diffusional clearance of the drug between the serosa and mucosa
- $CL_{gw}$ = intrinsic clearance by the gut

Thus, the fraction escaping gut wall clearance, $f_g$ (and therefore, $E_g$ and the intestinal organ clearance, $CL_{gw,org}$ from eq. 2), is a complex function of the blood flow to the organ, the diffusional clearance, and the intrinsic clearance of the drug. It is interesting to consider three limiting conditions of eq. 4.

Case 1: If there is no diffusional barrier ($CL_{diff} \gg CL_{gw}$)

$$f_g = 1$$

This is the situation where there is a perfusion rate-limited distribution of the drug, and the diffusional clearance has no effect on the extraction ratio. This equation is essentially the same as that for the well-stirred model of the liver.

Case 2: If there is a diffusional barrier at the mucosa-serosa interface ($CL_{diff} \ll Q$)

$$f_g = \frac{Q}{Q + CL_{gw}}$$

In such a case, there is a diffusion rate-limited distribution of drug and no extraction results.

Case 3: For a drug with high intrinsic clearance and poor diffusion ($CL_{gw} \gg CL_{diff}$)

$$f_g = \frac{Q}{Q + CL_{gw}}$$

All statistical analyses involved analysis of variance and were conducted with StatView (version 4.01; SAS Institute, Cary, NC).

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In such a case, there is a diffusion rate-limited distribution of drug and no extraction results.

Case 3: For a drug with high intrinsic clearance and poor diffusion ($CL_{gw} \gg CL_{diff}$)

$$f_g = \frac{Q}{Q + CL_{gw}}$$
The above model highlights the potential importance of diffusional barriers in intestinal extraction. Cases 2 and 3 clearly display situations where a large extent of extraction is determined by the diffusional clearance between the mucosal and serosal spaces.

**Diffusional Clearance of Phenol.** After vascular dosing in the diffusional model, the AUC to infinity in any compartment yielded the intrinsic clearance, $CL_{gw}$ (Appendix IB).

$$\text{AUC}_m = \text{AUC}_c = \frac{D}{CL_{gw}}$$  \hspace{1cm} (5)

The above model highlights the potential importance of diffusional barriers in intestinal extraction. Cases 2 and 3 clearly display situations where a large difference between pre- and postabsorptive elimination might result from the existence of a diffusional barrier at the serosa-mucosa interface.

**STELLA Simulations.** To get a better understanding of the metabolite kinetics of phenol, simulations were done with STELLA simulation software (Macintosh version 4.0; HPS Systems, Hanover, NH). An initial model (Model I) was built that assumed the gut to be a single compartment. Because this did not satisfactorily simulate the experimental results, a more complex model (Model II) was built further dividing the gut wall based on the cellular compartmentalization of intestinal enzymes. The schematics of the models used are provided in Figs. 3 and 4.

**Results**

**Viability.** The perfusate pressure at the SMA and the portal $pO_2$ were used to assess viability. For both drugs, a standard deviation of not more than 5% from the mean was seen during the course of the experiment (Kothare, 2001), indicating viability of the preparations. In the studies with different flow rates, the SMA pressure showed a rising trend with increasing flow rate, indicating an increasing resistance within the blood vessels. The $pO_2$ levels also increased with flow, indicating more anaerobic conditions presumably due to a shorter residence time.

**Vascular Dosing.** Table 1 summarizes the postabsorptive intestinal metabolism of phenol. A low "postabsorptive" extraction ratio was obtained after vascular dosing for phenol, and there was no statistical difference between the extraction ratios obtained at the high and low vascular doses. Figure 5 shows the concentration-time profiles of phenol at the low vascular dose. The phenol concentration in the reservoir declined in a biphasic manner. The higher vascular dose of phenol revealed a similar pattern (Kothare, 2001).

The two metabolites of phenol showed different shapes in the concentration-time curves (Fig. 5). The sulfate appeared early and then reached an asymptotic value. The glucuronide, on the other hand, appeared later and ascended slowly. An analysis of the luminal secretions showed that neither phenol nor the conjugates were secreted to a large extent (<1% of dose) (Kothare, 2001).

At the high vascular dose of phenol, the portal AUC ratio (sulfate/phenol), 0.30 ± 0.06, was statistically greater ($p < 0.05$) than the portal AUC ratio (glucuronide/phenol), 0.13 ± 0.02. Similarly, at the low vascular dose, the portal AUC (sulfate/phenol), with a value of 0.29 ± 0.14, statistically exceeded the portal AUC ratio (glucuronide/phenol) at 0.02 ± 0.02. This confirmed sulfation as the predominant metabolic pathway after vascular dosing.

**Luminal Dosing.** In sharp contrast to the vascular dosing case, the low luminal dose of phenol was more extensively metabolized, as evidenced by comparing the concentration-time profiles (Fig. 6) and the value of the AUC ratio (metabolite/phenol) from each route to those in Fig. 5. At the low luminal dose of phenol, the intestinal intrinsic clearance approached the perfusate flow rate (Table 2). Glucuronidation was the predominant metabolic pathway in the majority of rats, with the glucuronide levels exceeding those of the sulfate at most time points. At the low luminal dose of phenol, the portal AUC ratio (glucuronide/phenol) was 1.18 ± 0.02 whereas the portal AUC ratio (sulfate/phenol) was 0.74 ± 0.35. The AUC ratio (metabolite/phenol) overall exceeded that after vascular dosing, indicating greater metabolism.

The distinct metabolic profiles seen with vascular dosing of phenol were repeated with the luminal dose (Fig. 6). Phenyl sulfate again reached an asymptote. In contrast, phenyl glucuronide continued its ascending trend even at later time points when most of the phenol (the assumed driving force) was all but gone. This trend was repeated in the concentration-time profiles at the higher luminal dose of phenol as...
well (Kothare, 2001). However, at the high luminal dose, the intrinsic intestinal clearance was reduced by half, indicating saturation of metabolism (Table 2). At the higher luminal dose of phenol, the portal AUC ratio (glucuronide/phenol) was 0.18 whereas the portal AUC ratio (sulfate/phenol) was 0.15. Both routes showed a statistically lower AUC ratio (metabolite/phenol) at the higher luminal dose, indicating that saturation was occurring in both pathways.

Based on eq. 6, the mean diffusional clearance ($CL_{\text{gw}}$) of phenol could be calculated. Because $CL_{\text{gw}}$ was determined from luminal dosing and the AUC was determined after vascular dosing, it was necessary to use the doses from the two routes of administration that generated similar “effective” concentrations at the enzyme site. Keeping this in mind, the high vascular dose of phenol ($2000 \mu g$), when divided by the reservoir volume (200 ml), yielded an effective concentration of $10 \mu g/ml$. The low luminal dose ($100 \mu g$), when divided by the rat intestinal volume of $11 ml$ for a 250 g rat (Davies and Morris, 1993), yielded a concentration of $9 \mu g/ml$. The initial effective concentrations generated by the high vascular dose and the low luminal dose were, therefore, comparable. The diffusional clearance ($CL_{\text{gw}}$) of phenol was $1.11 ml/min$, which was much smaller than the perfusion flow rate. This is similar to Case 2 (eq. 4b) and is a possible explanation for the negligible postabsorptive extraction seen.

**Effect of Flow Rate on Clearance.** The results of varying the flow rate are summarized in Table 3. A Fisher’s post hoc analysis revealed that the postsorbptive extraction ratio, $E_{gw}$, at the lowest flow rate (5 ml/min) was significantly greater than that at each of the higher flow rates ($p < 0.05$). The gut extraction ratios at each of the higher flow rates (7.5–12 ml/min) were statistically similar to each other.

### Table 1

<table>
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<tr>
<th>Dose (µg)</th>
<th>CL$_{gw}$,org (ml/min)</th>
<th>E$_{gw}$</th>
<th>n</th>
<th>S.D.</th>
</tr>
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<td>1462 ± 285</td>
<td>0.66 ± 0.16</td>
<td>0.09 ± 0.02</td>
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</tr>
<tr>
<td>2513 ± 239</td>
<td>0.84 ± 0.09</td>
<td>0.11 ± 0.01</td>
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</table>

### Table 2

<table>
<thead>
<tr>
<th>Dose (µg)</th>
<th>n</th>
<th>CL$_{gw}$ (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>116 ± 16</td>
<td>4</td>
<td>7.29 ± 1.39$^a$</td>
</tr>
<tr>
<td>1044 ± 238</td>
<td>3</td>
<td>3.55 ± 1.16$^a$</td>
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</table>

$^a$ A one-way analysis of variance indicated that the intrinsic clearance was significantly higher at the low luminal dose, $p < 0.05$.  

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**Fig. 5.** Concentration-time profiles of phenol (■), phenyl glucuronide (○), and phenyl sulfate (▲) after the low vascular dose of phenol from the reservoir and the portal samples. Data are represented as mean ± S.D. of $n = 3$.

**Fig. 6.** Concentration-time profiles of phenol (■), phenyl glucuronide (○), and phenyl sulfate (▲) after the low luminal dose of phenol from the reservoir and the portal samples. Data are represented as mean ± S.D. of $n = 3$.
intestinal organ clearance, CLgw,org a at 5 ml/min was also significantly lower than that at each of the higher flow rates. No obvious trends in the phenol concentration-time profiles were seen (Kothare, 2001). The AUC ratio (metabolite/phenol) at each of the flow rates showed that sulfonation remained the major metabolic pathway at each of the flow rates and there were no obvious flow-dependent trends in the overall formation of the metabolite (Kothare, 2001). However, a comparison of the glucuronide concentration-time profiles from the reservoir samples (Fig. 7) revealed that there was a greater lag time in the appearance of the glucuronide at 5 ml/min compared with each of the higher flow rates.

**Stella Modeling.** There is no evidence in the literature to suggest sequential metabolism for either of the conjugates of phenol in the rat. Therefore, in a recirculating system, as long as substrate is present the conjugates should display a biphasic accumulation curve (an initial rapid formation phase followed by subsequent slower accumulation phase). This was not observed for either of the metabolite concentration-time profiles. In addition, concentration-time profiles of the two conjugates showed distinctly different shapes.

**Stella simulation software** was used to get a better mechanistic understanding of the metabolite kinetics. Simulations were conducted for the vascular and luminal dosing case; however, only the luminal dosing case is discussed here. The equations used to set up the models will be provided by the authors upon request (also published in Kothare, 2001). An initial model, termed “Model I” (Fig. 3), assumed that formation was the rate-limiting step in the appearance of the conjugates in the perfusate. As expected, the simulated reservoir concentration-time profile yielded a biphasic accumulation curve (Fig. 8) in contrast to the observed reservoir concentration-time profile (Fig. 6). Therefore, the more complicated Model II (Fig. 4) was developed. The gut wall was divided into cytosolic and ER compartments. The simulated reservoir concentration-time profiles now generated (Fig. 9) were more consistent with the experimentally observed profiles in Fig. 6. Also, perhaps more interestingly, the concentration-time profile of the glucuronide in the various compartments (Fig. 10) showed a significant accumulation of the glucuronide within the ER lumen. Thus, the simulations suggested a rate-limiting step involved in the efflux of the glucuronide as well as a metabolic recycling of the sulfate.

**Discussion**

This study aimed to experimentally evaluate the role of enzyme localization in pre- and postabsorptive intestinal elimination. A thorough theoretical assessment of a diffusional model of intestinal elimination was conducted. Alternate models, including those based on the segregation of intestinal blood flow, have also been evaluated and will be published in due course.

The postabsorptive intestinal extraction ratio of phenol was very small. It was indeed much lower than the values reported in the literature after intraduodenal dosing of phenol (Cassidy and Houston, 1984). After vascular dosing, the drug apparently has limited access to the apically expressed metabolizing enzymes. From a diffusional perspective, the effective path length of a vascularly administered drug molecule to the enzyme site would be magnified severalfold due to the tortuosity of its pathway from the vasculature to the villus tip. As a result, its “effective diffusivity” after vascular administration could be much lower than that after luminal administration.

A theoretical analysis revealed that after vascular dosing, the fraction escaping gut wall metabolism (as well as the intestinal organ clearance) was a complex function of intestinal blood flow, intrinsic clearance of the drug by the gut, and the diffusional clearance of the drug between the serosa and the mucosa. The calculated diffusional clearances of phenol were much smaller than the intestinal blood flow. In such a situation, the theoretical model predicted that the fraction escaping gut wall metabolism would be unity. Indeed, the experimental results showed negligible postabsorptive intestinal extraction ratios. In other words, there was a diffusional barrier to the metabolism of the vascularly administered drug at the mucosa-serosa interface.

Because the vascular blood flow is also an important determinant of

**Table 3**

<table>
<thead>
<tr>
<th>Q (ml/min)</th>
<th>Dose (µg)</th>
<th>CLgw,org (ml/min)</th>
<th>Epw (µg/ml/min)</th>
</tr>
</thead>
<tbody>
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<td>0.14 ± 0.02</td>
</tr>
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<td>7.5</td>
<td>2513 ± 239</td>
<td>0.84 ± 0.09</td>
<td>0.11 ± 0.01</td>
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<td>2546</td>
<td>1.04</td>
<td>0.11</td>
</tr>
<tr>
<td>12</td>
<td>4272</td>
<td>1.29</td>
<td>0.11</td>
</tr>
</tbody>
</table>

*Data expressed as mean ± S.D. (n = 3).

The intestinal clearance at 5 ml/min was statistically lower than that at each of the higher flow rates as determined by a Fisher’s post hoc analysis (p < 0.05).

The extraction ratio at 5 ml/min was statistically greater than that at each of the higher flow rates as determined by a Fisher’s post hoc analysis (p < 0.05).

Data are expressed as mean ± S.D. (n = 3) at flow rates of 5 (■) and 7.5 (○) ml/min; mean (n = 2) at flow rates of 10 (△) and 12 (●) ml/min.
intestinal clearance, the effect of changing vascular flow rate on intestinal organ clearance was evaluated. The lowest flow rate of 5 ml/min yielded statistically greater extraction than the higher flow rates, presumably due to a greater residence time within the tissue, but the extraction ratio was still very low. Because the diffusional clearance calculated was much smaller than the vascular flow rate, Case 2 (eq. 4b) of the theoretical model was applicable and predicted that the entire dose would escape gut wall metabolism. As predicted, changes in blood flow had little effect on the extraction ratio. The lowest flow rate was associated with a greater lag time in the appearance of the glucuronide compared with the higher flow rates.

In marked contrast to the vascular dosing case, after the low luminal dose of phenol, the concentrations of phenol approached zero by the end of the experiment. Moreover, the AUC ratios (metabolite/phenol) after luminal dosing exceeded those observed after vascular dosing. In addition, the intrinsic gut clearance at the low dose approached the intestinal blood flow, qualifying phenol as a high clearance drug (Cassidy and Houston, 1984).

Similar differences between luminal and vascular dosing were reported earlier from this lab (Wen et al., 1999) with 6-aminocarbavir (6AC), a prodrug of the carbocyclic nucleoside, carbavir. The conversion of 6AC to carbavir is carried out by adenosine deaminase (Chinsky et al., 1990). Both the 6AC and the phenol examples clearly refute the premise of the intestine as a well stirred organ; hence, models of intestinal extraction contingent on a diffusion rate-limited distribution of drug seem more appropriate. From a practical standpoint, the loss of this heterogeneity in disruptive systems like cell layers, enterocytes, and microsomes probably contributes to their frequent failure to predict in vivo intestinal clearance.

Two aspects of the metabolite kinetics of phenol were notable—the distinct shapes of each of the metabolite curves and the route dependence of the formation of the primary metabolite. Because the literature does not suggest sequential metabolism of either conjugate, these curves may be more reflective of enzyme localization at the cellular level. In theory, the plateau observed with the sulfate reservoir concentration profile could indicate either a saturation in its formation or a metabolic recycling to the parent. Sulfate ions were constantly supplied to the intestine by way of the Krebs-Henseleit buffer in the vascular perfusate; hence, a depletion of the cosubstrate seems unlikely. Intracellular metabolic recycling to the parent phenol might explain the plateauing of the sulfate curve. In fact, the reservoir sulfate profiles showed a double peak effect, which would be in keeping with such a recycling.

After luminal administration, the glucuronide concentrations continued to rise even as phenol concentrations (the presumed driving force) approached zero. Furthermore, there was a decrease in AUC ratio (metabolite/phenol) and intrinsic clearance in going from the low to high luminal dose. This may indicate a saturable process being involved in the release of the glucuronide from the cell.

STEELA simulation software was used to gain a better understanding of the metabolite kinetics of phenol. Model I assumed formation rate-limited metabolism kinetics in a “closed” recirculating system. Predictably, the metabolites showed biphasic ascending concentration curves, unlike the experimentally observed results.

Thus, the more complicated Model II was developed. This divided the gut wall based on the cellular localization of enzymes into cytosolic and membrane (endoplasmic reticulum)-bound compartments. Conceivably, phenol entering the gut epithelium would have immediate access to the cytosolic PSTs but would have to diffuse across the lipophilic ER membrane to access the UGTs. The sulfate formed could either recirculate unchanged or metabolically recycle to the parent phenol (Tan and Pang, 2001). Metabolic (“futile”) recycling of the sulfate could explain the plateau observed in its concentration-time profile. Phenol’s access to the UGTs in the ER lumen was modeled as a diffusional process. By setting the diffusional clearance of the glucuronide to be smaller than its formation clearance, a diffusional barrier to the glucuronide was modeled. The simulated reservoir glucuronide profile (Fig. 9) now more closely resembled the experimental observations. Interestingly, this resulted in a significant accumulation of the glucuronide within the ER lumen. This could explain the continued appearance of the glucuronide despite the declining phenol concentration.

A putative rate-limiting step in intestinal glucuronidation release was also suggested by a study with salicylamide (Barr and Riegelman, 1970). Salicylamide was administered in vitro to rabbit everted gut sacs and by in vivo perfusion of an intestinal loop with collection of mesenteric vein effluent. In the in vitro experiments, whereas the salicylamide dose was increased severalfold, the amount of glucuronide formed remained constant. Furthermore, in the in vivo experiments, there was an appearance of the glucuronide even though the salicylamide concentrations were declining. With the recognition that a member of the multidrug resistance-associated protein family is expressed in the intestine (Peng et al., 1999; Mottino et al., 2000), the capacity-limited efflux of the glucuronide seems reasonable.

The striking “route” dependence in the major metabolic pathway is intriguing. Conceivably, phenol readily encountered the cytosolic PSTs but required time to diffuse across the lipid-rich barrier of the ER to access the active site of the UGTs. The phenyl glucuronide, being more polar and much larger than its aglycone, would experience...
an even greater diffusional barrier to its efflux from the ER lumen. Therefore, sulfation would be expected to be the primary metabolic pathway, as seen after vascular administration. In sharp contrast, luminal administration of phenol yielded the glucuronide as the primary metabolite, in addition to a much greater overall extraction. This could be indicative of greater and perhaps more prolonged access of the perorally administered phenol to the apically placed UGTs. Thus, models of the intestine that are adequate to model intestinal clearance. The mucosal compartment at the cellular and tissue level on intestinal clearance, the division of the intestine into the lumen, mucosa, and serosa might in itself not be limited distribution of compounds need further investigation.

In conclusion, in light of the clear influence of enzyme localization at the cellular and tissue level on intestinal clearance, the division of the intestine into the lumen, mucosa, and serosa might in itself not be adequate to model intestinal clearance. The mucosal compartment could be further divided into a compartment for membrane-bound enzymes like the UGTs and CYPs and another for cytosolic enzymes like the sulfotransferases. Thus, models of the intestine that are contingent on a diffusion rate-limited rather than perfusion rate-limited distribution of compounds need further investigation.

Appendix IA: Effect of a Diffusional Barrier on Intestinal Clearance After Vascular Dosing

Based on the model depicted in Fig. 2, the following mass transfer equations defined the rates of change of drug amount in each compartment after vascular dosing.

In the reservoir:
\[ \frac{dX_r}{dt} = Q \cdot C_r - Q \cdot C_s \]  
(A-1)

In the serosal compartment:
\[ \frac{dX_s}{dt} = Q \cdot C_s - CL_{\text{diff}} \cdot C_s + CL_{\text{diff}} \cdot C_m - Q \cdot C_s \]  
(A-2)

In the mucosa:
\[ \frac{dX_m}{dt} = CL_{\text{diff}} \cdot C_s - CL_{\text{diff}} \cdot C_m - CL_{gw} \cdot C_m \]  
(A-3)

In the above equations:
- \( X_r \) = amount of drug in the reservoir,
- \( X_s \) = amount of drug in the serosa,
- \( X_m \) = amount of drug in the mucosa,
- \( Q \) = perfusate flow rate,
- \( C_s \) = concentration of drug in the serosa,
- \( C_r \) = concentration of drug in the reservoir,
- \( C_m \) = concentration of drug in the mucosa,
- \( CL_{\text{diff}} \) = diffusional clearance of drug between the mucosa and serosa,
- \( CL_{gw} \) = intrinsic clearance of the drug from the mucosa.

Integrating the above equations with respect to time from 0 to \( \infty \), eqs. A-4 through A-6 are obtained:
\[ Q \cdot AUC_s - Q \cdot AUC_r = -D \]  
(A-4)
\[ (-Q - CL_{\text{diff}}) \cdot AUC_s + Q \cdot AUC_r + CL_{\text{diff}} \cdot AUC_m = 0 \]  
(A-5)
\[ CL_{\text{diff}} \cdot AUC_r + (-CL_{\text{diff}} - CL_{gw}) \cdot AUC_m = 0 \]  
(A-6)

In the above equations

\[ AUC_s = AUC_r = AUC_m = AUC \text{ of drug in the serosa,} \]
\[ AUC_r = AUC \text{ of drug in the reservoir,} \]
\[ AUC_m = AUC \text{ of drug in the mucosa.} \]

Equations A-4 through A-6 were solved simultaneously to yield explicit expressions for \( AUC_s \) and \( AUC_r \):
\[ AUC_s = \frac{D \cdot (CL_{\text{diff}} + CL_{gw})}{CL_{\text{diff}} \cdot CL_{gw}} \]  
(A-7)
\[ AUC_r = \frac{D \cdot (CL_{\text{diff}} \cdot CL_{gw} + CL_{\text{diff}} \cdot Q + CL_{gw} \cdot Q)}{CL_{\text{diff}} \cdot CL_{gw} \cdot Q} \]  
(A-8)

Since the instantaneous extraction ratio, \( E_{gw} \), across the intestine after vascular dosing is defined as
\[ E_{gw} = 1 - \frac{C_s}{C_r} \]  
From 0 to \( \infty \) this may be expressed as
\[ E_{gw} = 1 - \frac{AUC_s}{AUC_r} \]  
(A-9)

The fraction escaping gut wall metabolism, \( f_q \), is therefore:
\[ f_q = \frac{Q \cdot (CL_{\text{diff}} + CL_{gw})}{CL_{\text{diff}} \cdot CL_{gw} + CL_{\text{diff}} \cdot Q + CL_{gw} \cdot Q} \]  
(A-11)

Appendix IB: Effect of a Diffusional Barrier on Intestinal Clearance After Luminal Dosing

From the model in Fig. 2, the rate of change of drug after luminal dosing in the reservoir and serosal compartment is still described by eqs. A-1 and A-2. The rate of change of drug in the mucosa after luminal dosing is given in eq. A-3 and is given in eq. A-16:
\[ Q \cdot AUC_s - Q \cdot AUC_r = 0 \]  
(A-13)
\[ (-Q - CL_{\text{diff}}) \cdot AUC_s + Q \cdot AUC_r + CL_{\text{diff}} \cdot AUC_m = 0 \]  
(A-14)
\[ CL_{\text{diff}} \cdot AUC_r + (-CL_{\text{diff}} - CL_{gw}) \cdot AUC_m = D \]  
(A-15)

The above equations were solved simultaneously to obtain the AUC in each compartment. The AUC in each compartment was exactly the same and is given in eq. A-16:
\[ AUC_m = AUC_r = AUC_s = \frac{D}{CL_{gw}} \]  
(A-16)

References


