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Effect of Flow on First-Pass Metabolism of Drugs: Single Pass Studies on 4-Methylumbelliferone Conjugation in the Serially Perfused Rat Intestine and Liver Preparations[1]

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ABSTRACT

The vascularly perfused rat intestine and liver preparations were used to examine the effect of flow (8 and 10 ml/min) on the sequential metabolism of 4-methylumbelliferone (4MU), which forms primarily the glucuronide conjugate (4MUG) in intestine and the sulfate conjugate (4MUS) in liver at low input concentrations of 4MU. In this system, a constant tracer concentration of [^{3}H]4MU was delivered systemically at 8 or 10 ml/min to the perfused rat small intestine preparation; the portal venous outflow perfusate at 8 and 10 ml/min was collected at steady state, reoxygenated and in turn delivered to the perfused rat liver preparation from a second rat donor. The intestinal extraction ratio and formation of 4MUG were decreased from 0.57 ± 0.07 to 0.49 ± 0.06 and 42 ± 5 to 36 ± 4% input rate, respectively, upon increasing the flow rate from 8 to 10 ml/min ($P < .05$). These decreases were the result of the reduction in transit time with increasing flows. In contrast, hepatic 4MU conjugation was increased (from 40 ± 7% to 48 ± 6% input rate to intestine) upon increasing the flow rate from 8 and 10 ml/min ($P < .05$), attributed primarily to increased formation of the major metabolite, 4 MUS, in liver (from 35 ± 9% to 39 ± 9% input rate to intestine). The unusual observation on increased hepatic metabolite formation with increasing flow could be rationalized. With increased flow to the serially perfused organs, there was an increased supply of substrate to the liver, the posterior organ, because of a faster intestinal transit time. Decreased intestinal metabolism (formation of 4MUG) at increased flow was compensated by increased hepatic metabolism (formation of 4MUS), albeit attenuated because of a faster hepatic transit time. The proportions of total 4MU conjugates formed (4MUG + 4MUS) across the intestine and liver remained constant at both flow rates. Hence, a rather constant overall extraction ratio (0.98 ± 0.004 and 0.97 ± 0.005, $P > .05$) existed across the two organs. The results demonstrate that the intestine, the anterior organ, plays a regulatory role on substrate supply to the posterior organ, the liver. With an increase in flow, the contribution of the intestine will decrease, whereas the contribution of the liver will increase in the overall first-pass metabolism.

The effect of flow on substrate removal in the liver has been widely studied. A reduction in flow normally brings about a more prolonged transit of drug through an eliminating organ, promoting efficient uptake and enhancing elimination, unless the flow rate and its accompanying pressure are insufficient in supporting full opening of the hepatic vasculature (Brauer *et al*., 1956; Pang *et al*. 1988). Conversely speaking, a faster rate of flow hastens drug transit and reduces drug extraction (Shand *et al*., 1975; Pang and Rowland, 1977a; Wilkinson, 1987). These have been found to occur for substrates such as oxyphenbutazone (Whitsett *et al*., 1971), lidocaine (Pang and Rowland, 1977b) and meperidine (Ahmad *et al*., 1983).

By contrast, the effect of flow on metabolite formation in the liver is rarely explored (Pang and Rowland, 1977c; Dawson *et al*., 1985). It is recognized that the metabolism of substrates is greatly modulated by acinar localization of enzymatic systems, that the anteriorly distributed system regulates the flux of substrate flowing downstream, a phenomenon exemplified in the conjugation of phenolic substrates (Morris and Pang, 1987). Under constant flow conditions, the proportion of sulfate formed decreases with increasing concentration, accompanied by compensatory increased glucuronidation. Saturation of the anterior, higher affinity sulfoconjugation system will render a disproportionately higher substrate flux reaching downstream for glucuronidation (Pang *et al*., 1981, 1983). When the reduction in sulfation is fully compensated by increased glucuronidation, the overall extraction ratio, E, will remain apparently constant despite

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ABBREVIATIONS: SMA, superior mesenteric artery; PV, portal vein; HV, hepatic vein; I, intestine; L, liver; TLC, thin layer chromatography; 4MU, 4-methylumbelliferone; 4MUS, 4-methylumbelliferyl sulfate; 4MUG, 4-methylumbelliferyl glucuronide; SGOT, serum glutamic-oxaloacetic transaminase.

that shifts in conjugation have occurred. The observation was first noted for harmol (Pang *et al*., 1981), then for other phenolic substrates: gentisamide, salicylamide and 4MU (Morris *et al*., 1988; Xu *et al*., 1990; Ratna *et al*., 1993).

With the realization that increased organ flow shortens the transit time of drug molecules and effectively reduces metabolism, Pang and Mulder (1990) explained the data of Dawson *et al*. (1985) which showed a reduction in harmol sulfation but unchanged glucuronidation (expressed in relation to the input rate to liver) with increasing flow to the liver. Constancy in the proportion of harmol undergoing glucuronidation downstream was caused by two diametrically opposing flow effects: substrate supply and transit time. With increased flow, an increased substrate flux spared from reduced sulfation now advanced downstream for glucuronidation, but, at the same time, the transit time within the downstream region was decreased. When these factors counterbalanced each other, the proportion of harmol undergoing glucuronidation would remain unchanged with increasing flow.

The effect of flow on the removal of drugs in first-pass organs has yet to be examined. The intestine and liver are two serially arranged first-pass organs known to possess metabolic activities. The anatomical placement of the intestine (anterior) and liver (posterior) may be viewed analogous to sulfation and glucuronidation, being upstream and downstream, respectively, in the liver. The purpose of the present work was to develop a meaningful vascular perfusion system for examination of flow effects on intestinal and hepatic metabolism. 4MU was chosen as the model substrate because it is conjugated appreciably, forming primarily 4MUG by the intestine [intestinal extraction ratio, E_I, varying from 0.36 to 0.6 (Mulder *et al.*, 1984; Zimmerman *et al.*, 1991)] but predominantly 4MUS in liver at low input concentrations (Ratna *et al*., 1993). The hepatic extraction ratio (E_H) is high (0.92–0.97) (Mulder *et al.*, 1985; Zimmerman *et al*., 1991; Ratna *et al*., 1993). Tracer [^{3}H]4MU concentration was used for the present studies such that the presence of 4MUG reflects mostly intestinal metabolism, and 4MUS, liver metabolism. The known futile cycling between 4MUS and 4MU (El-Mouelhi and Kauffman, 1986; Ratna *et al*., 1993) was assumed to be nonexistent in view of recently obtained high K_m value (760 μM) obtained for 4MUS desulfation from *in vitro* incubations (Chiba and Pang, unpublished data). Drawing on the analogy to harmol conjugation in liver (Dawson *et al*., 1985; Pang and Mulder, 1990), we hypothesized that an increased flow rate to the intestine and liver will bring about decreased E_I and 4MUG formation, but no change in E_H or perhaps increases in hepatically formed 4MUS. If hepatic metabolism compensated totally for the loss in intestinal metabolism of 4MU within this serially arranged unit, the intestine-liver, no net change in the total removal (expressed in relation to the input rate to intestine) will exist across these organs. However, changes in the total formation rate of 4MUS and 4MUG from the intestine and liver are expected to result with variation in flow.

The perfused rat small intestine-liver preparation developed by Hirayama *et al*. (1989), who used one rat donor, was modified. In view of the present objective of the flow study, only the superior mesenteric artery/portal venous circulation was used to perfuse the intestine and liver. The hepatic artery was ligated because its circulation to the liver would complicate results on liver metabolism with the flow changes. In view of omission of the hepatic arterial circulation for oxygen carriage in the circuit, adequate oxygen supply to both the intestine and liver must be ensured. Alternate perfusion models were therefore developed for this purpose. The modification entailed two rat donors and reoxygenation of the portal venous perfusate. Viability and oxygen consumption rates of the various intestinal and liver preparations were first assessed.

Materials and Methods

Materials

Unlabeled 4MU, 4MUS and 4MUG were purchased from Sigma Chemical Co. (St. Louis, MO). [^{3}H]4MU (specific activity, 31.8 Ci/mmol) was synthesized by catalytic tritium exchange (New England Nuclear, Boston, MA, and ICN Radiochemicals, Irvine, CA) and purified by TLC (Mulder *et al*., 1985) and high-performance liquid chromatography (Zimmerman *et al*., 1991) before use. The radiochemical purity of [^{3}H]4MU was $>$ 97%.

Perfusion Apparatus and Perfusate

A TWO/TEN Perfuser (MX International, Aurora, CO) was used. Perfusate consisted of 20% of washed human red blood cells (Red Cross, Toronto, Canada), 3% dextran T40 (Pharmacia Fine Chemicals, Piscataway, NJ), 1% bovine serum albumin (25% in Tyrode's buffer, Sigma), 300 mg/dl glucose (50% dextrose, Abbott Laboratories, Limited, Montreal, Canada) in Krebs-Henseleit Bicarbonate solution buffered to pH 7.4. Perfusate was oxygenated in rotating reservoirs or with a home-built oxygenator (Lindros, 1974) with 95% O_2-5% CO_2 (fig. 1).

Intestine-Liver Perfusion

The surgical procedure for rat small intestine perfusion (Hirayama *et al*., 1989) and liver perfusion (Pang and Rowland, 1977b) have been described previously. Male Sprague-Dawley rats (240–313 g, Charles River, St. Constant, QC, Canada) were used as donors. Rats were abstained from food but not glucose (2% sugar solution) nor water for 24 h before study. The hepatic artery was ligated for the present flow studies, such that the same flow rate entering the intestine would also reach the liver. Two rats were used for each experiment, one as the intestine donor and the other as the liver donor. The weights of rat liver donors varied from 240 to 310 g, such that the accompanying liver weights were less than 8.6 g. With the designated flow rates (8 and 10 ml/min), the lowest hepatic flow rate was 0.93 ml/min/g, thus avoiding derecruitment of the liver which could occur at reduced flow rates ($<$0.75 ml/min/g) to the liver (Pang *et al*., 1988).

The IL preparation. In this IL preparation (fig. 1A), the outflow from the portal vein of the intestine was fed into a home-built oxygenator (Lindros, 1974) made up with a coil of gas-permeable silastic tubing (170 cm) encased in a sealed conical flask as an oxygen-exchange chamber (95% O_2-5% CO_2). Reoxygenated perfusate was then used to perfuse the liver *via* the portal vein of the second donor. The IL preparation was perfused in a recirculation manner for 60 min each for the two chosen flow rates (8 or 10 ml/min) for the determination of viability.

The alternate single pass I+L perfusion system. Because leakage of perfusate was associated with the backpressure of the oxygenator of the IL preparation due to the long tubing (see fig. 1A), and because intense sampling of the portal vein during the experiment would inevitably affect the flow rate to the liver, the perfusion circuit was modified further (fig. 1B). In the alternate I+L preparation, intestine perfusion was first conducted at 8, then 10 ml/min, each of 60-min duration with perfusate containing the same concentration of [^{3}H]4MU from reservoir 1 (R1). Portal venous outflow was

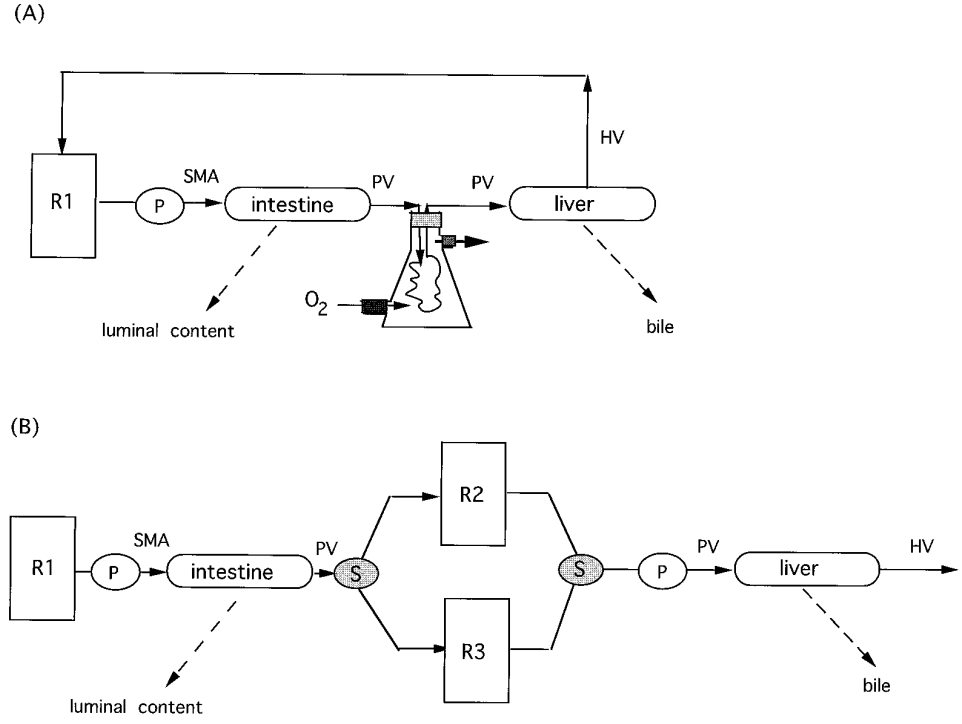

Fig. 1. Schematic representation of (A) IL preparation with the home-built oxygenator interposed between the intestine and liver, and (B) the serially perfused intestine and liver (I+L) preparation. The arrows indicate the direction of blood flow (*Q*); R1, R2 and R3 are reservoirs with oxygenation. P and S represent perfusion pumps and switches, respectively. SMA, PV and HV denote the catheter placement at the SMA, the PV and HV.

diverted, at steady state (20 min after commencement of perfusion period, predetermined in preliminary experiments), into two reservoirs (R2 and R3) for collection of the perfusate outflows from the intestine at flow rates of 8 and 10 ml/min. The liver of the second rat donor was perfused with the reoxygenated perfusate from R2 (8 ml/min outflow from intestine) then R3 (10 ml/min outflow from intestine) for 27 to 30 min each at 8 and 10 ml/min, respectively; the hepatic venous perfusate exited the liver *via* the hepatic vein. Bile was collected at 5- to 10-min intervals.

Tracer [^{3}H]4MU Experiments

A constant concentration of tracer [^{3}H]4MU (154–356 $\times$ 10^3 dpm/ml) was employed in R1 for intestinal perfusion. Three to four samples were taken from R1 at each flow rate, and the mean of these determinations was taken as C_{SMA} (the steady-state input concentration to the intestine). Five portal venous samples were collected during steady state (20–60 min) per perfusion period. Data so obtained were averaged for the steady-state outflow concentrations for the intestine C_{PV}. Luminal fluid was collected *in toto* for each flow rate, and the lumen was rinsed twice with saline; all washes and luminal fluids were pooled and the radioactivity quantified.

Perfusate from R2 and R3 were assayed for the respective input concentrations to the liver. Five hepatic venous samples were collected at 10 to 27 min and these determinations were averaged to provide the steady-state hepatic venous outflow concentration, C_{HV}. Bile usually was collected at 5-min intervals into pretared vials.

Viability of the IL Preparations

Pressure. Pressure at SMA was monitored at 5-min intervals throughout the entire perfusion period.

Oxygen consumption. The reservoir perfusate, portal venous perfusate (before and after oxygenation), and hepatic venous perfusate for the recirculating IL preparation were sampled for oxygen consumption determinations by an oxygen electrode (Instech Laboratories, Inc., Horesham, PA) and for other viability tests. Oxygen consumption rate by the intestine was estimated as the arteriovenous difference in oxygen tension after correction for the pH of the inflow and outflow perfusate. The oxygen consumption rate for each organ/tissue was estimated as follows:

$$O_2 \text{ consumption (ml/min)} = Q \cdot (O_{2,in} - O_{2,out}) \quad (1)$$

where $O_{2,in}$ and $O_{2,out}$ are the oxygen concentrations in perfusate immediately entering and exiting the organ, respectively, and Q is the blood perfusate flow rate. For the liver, $O_{2,in}$, the oxygen concentration is the concentration in the reoxygenated portal venous perfusate. The concentration of oxygen in blood perfusate was determined by the equation:

$$O_2 \text{ concentration (ml/100 ml)} = 0.003 \cdot pO_2 + 1.34 \cdot [Hb] \cdot O_2Sat\%/100 \quad (2)$$

where the degree of saturation (O_2Sat%), influenced by both the hemoglobin concentration ([Hb], g/100 ml) and pH, was read from a nomogram (Severinghaus, 1958). Hemoglobin concentration was determined spectrophotometrically at 540 nm (Uvikon 860, Kontron Instruments, Everett, MA) in 20-μl perfusate samples, with cyanmethemoglobin reagents as the standards (Stanbio Laboratory, Inc., San Antonio, TX).

Other viability tests. SGOT concentrations in portal venous perfusate plasma (from R2 and R3) and hepatic venous perfusate plasma were estimated by use of a kit (Sigma Diagnostics, St. Louis, MO). The time course of the bile production rate was expressed per gram of liver tissue. The weight of bile collected was converted to volume, assuming a density of one.

Quantitation of [^{3}H]4MU and Metabolites in Perfusate Blood, Plasma and Bile

[^{3}H]4MU in perfusate blood (0.5–2 ml) was quantified by an extraction procedure with ethyl acetate as described elsewhere (Zimmerman *et al.*, 1991). Standards consisting of varying known counts of [^{3}H]4MU were processed along with the samples for construction of a calibration curve for the correction of lack of complete recovery.

[^{3}H]4MU, [^{3}H]4MUG and [^{3}H]4MUS in plasma and bile were assayed by TLC (Mulder *et al.*, 1984), with appropriate corrections made for on-plate recoveries; an equal aliquot of the sample used for

TLC was subjected to liquid scintillation spectrometry (Beckman 5801, Mississauga, ONT). The solvent system was 1-butanol/ethanol/water, 6:2:2 (v/v/v). Silica Gel GF plates (5 × 20 cm, 250 μm, Analtech, Inc., Newark, DE) were spotted with authentic 4MU, 4MUS and 4MUG at the origin. Plasma (100 μl) or bile samples (50 μl, diluted with 200 μl water) were then applied onto the origin. After development, the bands corresponding to unlabeled 4MU, 4MUS and 4MUG which coeluted with their labeled counterparts were identified under UV light (R_f = 0.43, 0.75 and 0.90 for 4MUG, 4MUS and 4MU, respectively) and scraped into 6-ml poly Q vials; 0.5 ml of water and 5 ml of liquid scintillation fluor (Ready Safe, Beckman, CA) were added. After mixing, the vials were maintained in darkness for 24 h to reduce chemiluminescence before liquid scintillation spectrometry.

Calculation

All data were subsequently expressed as dpm/ml in blood perfusate for accountability of the recovery, inasmuch as [^{3}H]4MU concentrations in blood perfusate was used as an input to the SMA. Plasma concentrations were converted to blood concentrations by multiplication of the blood/plasma (C_B/C_P) partitioning ratio; these were (1-Hct) for 4MUS and 4MUG, where Hct is the hematocrit, because 4MUS and 4MUG were not distributed in red blood cells; for 4MU, C_B/C_P = 1.2 (Ratna *et al*., 1993). The steady-state extraction ratio of the intestine or liver was expressed as the difference between the steady-state inflow and outflow concentrations divided by the inflow concentration. The intestinal extraction ratio, E_I is:

$$E_I = \frac{C_{SMA} - C_{PV}}{C_{SMA}} \quad (3)$$

The intestinal availability, F_I is:

$$F_I = 1 - E_I \quad (4)$$

The hepatic extraction ratio, E_H is:

$$E_H = \frac{C_{PV} - C_{HV}}{C_{PV}} \quad (5)$$

The hepatic availability, F_H is:

$$F_H = 1 - E_H \quad (6)$$

The effective (total) extraction ratio, E_{tot}, across the first-pass organs, is:

$$E_{tot} = \frac{C_{SMA} - C_{HV}}{C_{SMA}} \quad (7)$$

The complement fraction, the overall availability, F_{tot}, across these organs, is given by the product of the availability of the intestine and the liver.

$$F_{tot} = \frac{C_{HV}}{C_{SMA}} = 1 - E_{tot} = F_I F_H = (1 - E_I)(1 - E_H) \quad (8)$$

The intestinal formation rate of the conjugate (primary metabolite mi), or v_I^{mi} could be assessed with the following equation:

$$v_I^{mi} = QC_{PV}\{mi\} + \Delta A_e^I\{mi\}/\Delta t \quad (9)$$

where $\Delta A_e^I\{mi\}/\Delta t$ is the luminal excretion rate of the conjugate, and $C_{PV}\{mi\}$ is the portal venous concentration of the conjugate. The rate of intestinal formation of each conjugate was further expressed as a percent of the input rate of 4MU to the intestine (QC_{SMA}).

The intestinally formed 4MU metabolites also entered the liver. The total output rate from the liver (summed hepatic venous rate output and biliary excretion rate) thus contained the intestinally formed metabolites. The assumption that little futile cycling had occurred for 4MU and 4MUS at tracer [^{3}H]4MU concentrations (El-Mouelhi and Kauffman, 1986; Ratna *et al*., 1993) was taken, such that the hepatic formation rate of each conjugate (primary metabolite mi), or v_H^{mi} could be assessed with the following equation (Xu *et al*., 1989):

$$v_H^{mi} = QC_{HV}\{mi\} + \Delta A_e^H\{mi\}/\Delta t - QC_{PV}\{mi\} \quad (10)$$

where $\Delta A_e^H\{mi\}/\Delta t$ is the biliary excretion rate of the conjugate, and $C_{HV}\{mi\}$ and $C_{PV}\{mi\}$ are the hepatic venous and portal venous concentration of the conjugate, respectively. The rate of hepatic formation of each conjugate may be further expressed as a percent of the input rate of 4MU to the intestine (QC_{SMA}) or as a percent of the input rate of 4MU to the liver (QC_{PV}).

The total I+L formation rate of each conjugate (primary metabolite mi), or v_{tot}^{mi} could be assessed with the following equation:

$$v_{tot}^{mi} = QC_{HV}\{mi\} + \Delta A_e^H\{mi\}/\Delta t + \Delta A_e^I\{mi\}/\Delta t \quad (11)$$

Upon summing the rates of conjugation for both conjugates (4MUS and 4MUG) according to equation 11, the total conjugation rate which represents the total first-pass metabolism may be obtained. The contribution of the intestine and liver to the formation of each conjugate was further expressed as a percent of the total rate of first-pass metabolism.

Recovery from the intestine was estimated as the summed output rate from the PV and luminal contents, divided by the input rate into the intestine (QC_{SMA}). Similarly, the recovery from the intestine and the liver was taken as the summed hepatic venous output rates, biliary excretion rates and luminal outflow rates of 4MUG, 4MUS and 4MU divided by the input rate of 4MU to the intestine, QC_{SMA}.

Statistics

Data were expressed as the mean ± S.D. A paired *t* test was used to compare the means, and a P value of 0.05 was considered significant.

Results

Viability of IL Preparations

The IL preparation. For this recirculating preparation, the pressure at SMA was 50 ± 2 mm Hg at 8 ml/min and 62 ± 2 mm Hg at 10 ml/min, and the values were constant throughout the perfusion period (table 1). The intestinal oxygen consumption rates were 0.80 ± 0.1 and 1.0 ± 0.1 μmol/min/g intestine at 8 and 10 ml/min, respectively, values similar to those reported by Hirayama *et al*. (1989). The perfusate volume at the end of recirculation was only 79 ± 8%, which suggests that there was appreciable loss caused by leakage (Hirayama *et al*., 1989). Liver oxygen consumption rate was lower (1.7 ± 0.1 and 1.9 ± 0.1 μmol/min/g liver for 8 and 10 ml/min, respectively) than 2 μmol/min/g liver. Bile flow rates, although constant throughout the perfusion period at both flow rates, were much lower than those reported for other liver preparations (Hirayama *et al*., 1989; St. Pierre *et al*., 1989; de Lannoy and Pang, 1993). SGOT concentrations in the reservoir perfusate plasma were less than 16 U/l throughout the entire perfusion period, which suggests integrity of the cellular membrane (table 1).

The alternate single pass serially perfused I+L system. Pressures at SMA were 38 ± 1.0 and 46 ± 1.8 mm Hg at 8 and 10 ml/min, respectively, and these remained constant throughout the entire perfusion period. These values were lower than those observed for the recirculating IL preparation for perfusion at the same flow rate. With reoxygenation of the portal venous outflow perfusate in the rotating reservoirs, oxygen consumption rates were increased dra-

TABLE 1

Summarized viability of intestine and liver preparations: the IL preparation for recirculation, and the serially perfused intestine and liver (I+L) preparation for single-pass perfusion

Function	IL Preparation[a]		I+L Preparation[b]	
	8 ml/min	10 ml/min	8 ml/min	10 ml/min
Intestinal				
Pressure at SMA (mm Hg)	50 ± 1.6	62 ± 1.5	38 ± 1.0*	46 ± 1.8*
Oxygen consumption rate (μmol/min/g)	0.8 ± 0.1	1.0 ± 0.1	1.1 ± 0.41	0.95 ± 0.2
Perfusate volume recovery (%)	79 ± 8.0		Not applicable	
Liver				
Oxygen consumption rate (μmol/min/g)	1.7 ± 0.1	1.9 ± 0.1	2.6 ± 0.96*	2.7 ± 0.7*
Bile flow rate (μl/min/g)	0.3 ± 0.1	0.5 ± 0.1	0.9 ± 0.08*	1.1 ± 0.01*
SGOT concentration (U/l)	<16	<16	<10	<10

[a] Values are mean ± S.D. for 2 h recirculating perfusion, $n = 5$. (Liver weights were 6.7 ± 0.6 g.)
[b] Values are mean ± S.D. for single-pass perfusion, $n = 3$. (Liver weights were 6.4 ± 0.3 g.)
* Data were significantly different from those of IL preparation; $P < .05$.

matically to 2.6 to 2.7 μmol/min/g. Bile flow rates were maintained constant and were considerably higher than those for the IL preparation; the values found were similar to those reported by others (St. Pierre *et al*., 1989; Ratna *et al*., 1993). SGOT concentration in hepatic venous perfusate plasma was less than 10 U/l throughout the perfusion period (table 1). The viability data showed that the I+L single-pass preparation was superior to the IL preparation for both intestine and liver functions.

Flow Effects on [^{3}H]4MU Intestinal and Hepatic Metabolism in the I+L Preparation

Intestinal extraction ratio and metabolite formation rate. Further studies were therefore conducted with the I+L preparation. Both 4MUS and 4MUG were observed in portal venous perfusate; 4MUG was the predominant metabolite, accounting for about 76% of total intestinal metabolism. The results were expected. The luminal contents accounted for less than 3.3% of the infused 4MU and contained 4MUG as the predominant species. With an increase in flow rate from 8 to 10 ml/min, the steady-state intestinal extraction ratio E_I of 4MU decreased from 0.57 ± 0.07 to 0.49 ± 0.06 ($P < .05$), with reduced formation of the major metabolite 4MUG (from 42 ± 5% to 36 ± 4% input rate of 4MU delivered to the intestine; $P < .05$) but virtually constant formation of 4MUS (13 ± 3% and 12 ± 3% input rate; $P > .05$) (table 2). The formation rates of 4MUS and 4MUG, expressed as percent input rate to intestine, decreased from 55 ± 7% to 48 ± 5% ($P < .05$) with the increase in flow rate.

Hepatic extraction ratio and metabolite formation rate. In hepatic venous outflow blood, 4MUS and 4MUG each accounted for about 43 to 55% of the total metabolites formed (table 3). Bile contained 4MU, 4MUS and 4MUG, and the total excretion rate accounted for 11 to 14% of the input rate of 4MU delivered to the intestine, with 4MUS as the predominant species. Upon summing the hepatic venous outflow rate and biliary excretion rate (expressed as percent of input rate to intestine) and subtracting the respective input rates of 4MUS and 4MUG entering the portal vein (from table 2), hepatic metabolite formation rates (equation 10) were obtained; 4MUS was the predominant metabolite formed in liver, accounting for 81 to 88% of total liver metabolism. When viewed with respect to the delivery rate of 4MU to the intestine, both 4MUS and 4MUG formation rates, hence the total hepatic conjugation rate, were increased ($P < .05$) from 35 to 39% and from 5 to 9%, respectively, when the flow rate increased from 8 to 10 ml/min.

When data were normalized with respect to the input rate of 4MU delivered to the liver, the steady-state hepatic extraction ratio E_H of 4MU (equation 5) was constant (0.943 ± 0.01 and 0.947 ± 0.01, $P > .05$) at both flow rates. Biliary excretion rates of 4MU and the 4MU conjugates were reduced at the higher flow rate. However, the proportions of 4MUS and 4MUG formed remained quite constant at both flow rates, rendering the total conjugation of 4MU unchanged (94–95%, $P > .05$) (table 4).

Total metabolism in the intestine and liver with flow changes. Upon addition of the output rates in luminal contents and bile to the output rate appearing in hepatic venous perfusate, the total conjugation rate by the intestine and liver, when expressed as percent input rate of 4MU to the intestine, was found to be constant (from 98 to 97%, $P > .05$; table 5). There was a small but statistical decrease in 4MUG but insignificant increase in 4MUS formation with increased flow. The effective extraction ratio E_{tot} across the intestine and liver (equation 7) remained constant at 0.98 ± 0.004 and 0.97 ± 0.004 ($P > .05$) at both flow rates. The overall availability F_{tot} equaled $F_I F_H$ according to equation 8.

The composite changes in intestinal and liver conjugation rates are depicted with respect to total first-pass conjugation (fig. 2). Accompanying the increment in flow rate from 8 to 10 ml/min, there was a significant decrease in the proportion of intestinal glucuronidation and compensatory increases in the proportion of hepatic sulfation and glucuronidation.

Discussion

Although the intestine of the IL preparation (fig. 1A) displayed adequate oxygenation consumption (*cf.* with 0.87 ± 0.38 μmol/min/g, Hirayama *et al*., 1989), the higher pressure at the SMA (because of backpressure created by the long tubing in oxygenator), the reduced hepatic oxygenation consumption rate (*cf.* 2.5–2.9 μmol/min/g; Hirayama *et al*., 1989; St. Pierre *et al*., 1989; deLannoy and Pang, 1993), decreased bile flow rate and the lack of conservation of reservoir volume rendered the combined preparation less desirable for exploration (table 1). The alternate, the serially perfused intestine and liver (I+L) preparation (fig. 1B), however, proved to be superior to the IL preparation. Leakage of blood was not observed, and SGOT levels were low; the pressure at the

TABLE 2

Effect of flow rate on formation of 4MU metabolites in the intestine of the serially perfused I+L preparation[a]

	Perfusate Flow Rate (Q)	
	8 ml/min	10 ml/min
Flow rate (ml/min/g)	1.3 ± 0.06	1.6 ± 0.07
4MU input concentration to intestine, C_{SMA} (dpm/ml)	243,000 ± 80,000	242,000 ± 74,000
4MU input rate to intestine, $Q\ C_{SMA}$ (dpm/min)	1,943,000 ± 642,000	2,423,000 ± 737,000
Extraction ratio (E_I)[b]	0.57 ± 0.07	0.49 ± 0.06*
Availability (F_I)[c]	0.43 ± 0.07	0.51 ± 0.06*
Portal venous outflow rate (% 4MU input rate to the intestine)		
4MUS	13 ± 3	12 ± 3
4MUG	41 ± 6	35 ± 4*
4MU	43 ± 7	51 ± 6*
Sum: 4MUS + 4MUG + 4MU	97 ± 3	98 ± 2
Luminal outflow rate[d] (% 4MU input rate to the intestine)		
4MUS	0.2 ± 0.06	0.1 ± 0.04
4MUG	1.7 ± 0.9	1.2 ± 0.5
4MU	1.4 ± 0.4	0.3 ± 0.1
Sum: 4MUS + 4MUG + 4MU	3.3 ± 0.6	1.7 ± 0.6
Intestinal metabolite formation rate[e] (% 4MU input rate to the intestine)		
4MUS	13 ± 3	12 ± 3
4MUG	42 ± 5	36 ± 4*
Sum: 4MUS + 4MUG	55 ± 7	48 ± 5
Recovery[f] (%)	99 ± 1	98 ± 1

[a] Steady-state data were expressed as mean ± S.D. of six experiments. (Intestines weights were 6.1 ± 0.3 g.)
[b] Equation 3.
[c] Equation 4.
[d] Luminal outflow rate was obtained by dividing the dpm in luminal fluid by the time of perfusion, and normalized by the input rate, $Q{\cdot}C_{SMA}$.
[e] Equation 9. (Data were expressed as percent of input rate to the intestine.)
[f] Sum of portal venous outflow and luminal outflow rates of 4MUS, 4MUG and 4MU, divided by the input rate of 4MU to the intestine.
* Data were significantly different from those at 8 ml/min; $P < .05$.

TABLE 3

Effect of flow rate on 4MU metabolite formation in rat liver of the serially perfused I+L preparation[a]

	Perfusate Flow Rate (Q)	
	8 ml/min	10 ml/min
Flow rate to liver (ml/min/g)	1.1 ± 0.14	1.4 ± 0.2
4MU input rate to intestine, $Q\ C_{SMA}$ (dpm/min)	1,943,000 ± 642,000	2,423,000 ± 737,000
(Total) hepatic venous outflow rate: QC_{HV} (% 4MU input rate to the intestine)		
4MUS	43 ± 3	49 ± 7*
4MUG	39 ± 5	38 ± 3
4MU	2.3 ± 0.5	2.6 ± 0.6
Sum: 4MUG + 4MUS + 4MU	85 ± 5	89 ± 7*
Biliary excretion rate (% 4MU input rate to the intestine)		
4MUS	9.8 ± 6.1	7.5 ± 6.2
4MUG	4.4 ± 1.5	3.0 ± 1.6*
4MU	0.2 ± 0.11	0.11 ± 0.07*
Sum: 4MUS + 4MUG + 4MU	14 ± 6	11 ± 7*
Summed hepatic venous outflow rate and biliary excretion rate (% 4MU input rate to the intestine)		
4MUS	53 ± 5	56 ± 4
4MUG	44 ± 5	41 ± 4
4MU	2.5 ± 0.4	2.7 ± 0.6
Sum: 4MUS + 4MUG + 4MU	99 ± 0.9	100 ± 0.6
Hepatic metabolite formation rate[b] (% 4MU input rate to the intestine)		
4MUS	35 ± 9	39 ± 9*
4MUG	5 ± 3	9 ± 3*
Sum: 4MUS + 4MUG	40 ± 7	48 ± 6*

[a] Steady-state data were expressed as mean ± S.D. of six experiments. (Livers weights were 7.3 ± 0.9 g.) Data were expressed as percent of rate to the intestine.
[b] Equation 10. (Data were expressed as percent of rate to the intestine.)
* Data were significantly different from those at 8 ml/min; $P < .05$.

SMA at 8 ml/min was similar to that reported by Hirayama *et al*. (1989) who used a comparable intestinal flow rate (7.5 ml/min). Moreover, the bile flow rate and oxygen consumption rate of this preparation were similar to those obtained by others (Hirayama *et al*., 1989; St. Pierre *et al*., 1989; deLannoy and Pang, 1993).

Use of this I+L serially perfusion system for hypothesis testing of flow effects on tracer [^{3}H]4MU intestinal glucuronidation and hepatic sulfation was thus appropriate. Data obtained at varying flow rates from the preparation indeed support this view. The placement of the organs/pathways and transit time considerations will influence the type of 4MU metabolites formed. Metabolism by the intestine and liver, two serially arranged organs, could be viewed conceptually analogous to sulfation (upstream) and glucuronidation (downstream), occurring sequentially within the liver: the intestine is analogous to the anterior sulfation system, and liver is analogous to the posterior glucuronidation system. The scenario is akin to anterior sulfation regulating the quantity of substrate for downstream glucuronidation (Pang and Mulder, 1990). Indeed, the presence of intestinal metabolism has been found to regulate the supply of substrates for hepatic elimination (Hirayama and Pang, 1990; Xu *et al*., 1989). When the effects of flow on upstream sulfation and downstream glucuronidation in liver were applied for postulating intestinal and liver metabolism (Pang and Mulder, 1990), two diametrically opposing factors should exist at the higher flow rate: substrate sparing from intestine metabolism and faster hepatic transit time. With increased flow rate, there will be reduced metabolism by the intestine, the anterior organ; metabolism by the liver, the posterior organ, will increase to compensate for the loss. The present data showed a reduction in E_I (table 2) but an unchanged E_H (table 4) with increased flow, a pattern which showed much similarity to harmol conjugation with flow: reduced sulfation but unchanged glucuronidation (Dawson *et al*., 1985). The reduction in E_I but unchanged E_H with increased flow is

TABLE 4

Effect of flow rate on 4MU metabolite formation in rat liver of the serially perfused I+L preparation[a]

	Perfusate Flow Rate (Q)	
	8 ml/min	10 ml/min
4MU input concentration to intestine, C_{PV} (dpm/ml)	104,500 ± 38,000	124,000 ± 45,000
4MU input rate to liver, $Q\ C_{PV}$ (dpm/min)	836,000 ± 304,000	1,240,000 ± 450,000
Extraction ratio (E_H)[b]	0.943 ± 0.011	0.947 ± 0.011
Availability (F_H)[c]	0.057 ± 0.01	0.053 ± 0.01
Biliary excretion rate (% input rate to the liver)		
4MUS	19 ± 11	12 ± 11*
4MUG	9 ± 3	5 ± 3*
4MU	0.4 ± 0.2	0.2 ± 0.1*
Sum: 4MUS + 4MUG + 4MU	28 ± 10	17 ± 13*
Hepatic metabolite formation rate[d] (% input rate to the liver)		
4MUS	81 ± 9	77 ± 8
4MUG	13 ± 8	18 ± 7
Sum: 4MUS + 4MUG	94 ± 1	95 ± 1

[a] Steady-state data were expressed as mean ± S.D. of six experiments. (Livers weights were 7.3 ± 0.9 g.)
[b] Equation 5.
[c] Equation 6.
[d] Equation 10. (Data were expressed as percent of rate to the liver.)
* Data were significantly different from those at 8 ml/min; $P < .05$.

TABLE 5

Effect of flow rate on 4MU metabolite formation across the intestine and liver[a]

	Perfusate Flow Rate (Q)	
	8 ml/min	10 ml/min
4MU input rate to intestine (dpm/min)	1,943,000 ± 642,000	2,423,000 ± 737,000
Extraction ratio (E_{tot})[b]	0.98 ± 0.004	0.97 ± 0.004
Availability (F_{tot})[c]	0.02 ± 0.004	0.03 ± 0.005
Metabolite formation rate[d] (% 4MU input rate to the intestine)		
4MUS	53 ± 5	56 ± 4
4MUG	45 ± 5	42 ± 4*
Sum: 4MUS + 4MUG	98 ± 0.4	97 ± 0.4
Recovery[e] (%)	101 ± 0.7	100 ± 0.2

[a] Steady-state data were expressed as mean ± S.D. of six experiments.
[b] Equation 7.
[c] Equation 8.
[d] Equation 11.
[e] Sum of total hepatic venous outflow rate, biliary excretion rate and luminal outflow rate of 4MUS, 4MUG and 4MU divided by the input rate of 4MU to the intestine, QC_{SMA}.
* Data were significantly different from those at 8 ml/min; $P < .05$.

expected, because increased flow will reduce the extraction ratios of moderate or poorly cleared compounds but is unable to affect the extraction ratios of highly cleared compounds (Pang and Rowland, 1977a). The total extraction across the intestine and liver, E_{tot}, which has a value close to unity, was also unchanged (table 5). When 4MU conjugation patterns were examined, 4MUG, the major intestinally formed metabolite, and total intestinally derived metabolites were both reduced at the higher flow rate, as expected for hastened drug transit time (table 2). By contrast, all hepatically

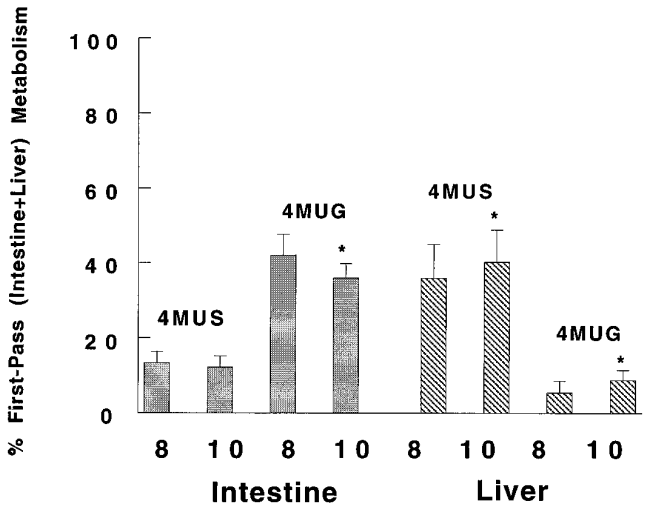

Fig. 2. Effect of flow rate on intestinal (shaded) and hepatic (hatched) formation of 4MUS and 4MUG. Metabolic data (mean ± S.D., $n = 6$) were expressed as percents of total (intestinal and liver) conjugation rate at the flow rates of 8 and 10 ml/min. Data at 10 ml/min were compared with data at 8 ml/min, and the asterisk indicates a P value of $< .05$.

formed metabolites, 4MUS and 4MUG as well as the proportion of total hepatic conjugation rate, were all increased (table 3, fig. 2), when data were expressed in terms of the input rate to the intestine or total first-pass metabolism. Yet, the total conjugation rate across the two organs remained constant at both flow rates (table 5).

When hepatic conjugation rates were alternatively expressed in terms of the input rate of 4MU to the liver (table 4), unchanged conjugation rates for 4 MUS and 4 MUG were found. The faster transit time with increased flow should have brought about proportionally reduced sulfation and increased glucuronidation within the liver. This was observed, though the changes failed to reach statistical significance (table 4).

The study above exemplified the interaction between flow and the intestine and liver, organs which are arranged serially, in first-pass metabolism. Increased perfusion flow to the intestine and liver had led to a reduced contribution of the intestine but increased hepatic contribution to first-pass metabolism (fig. 2). At lower perfusion flow to the intestine and liver, the contribution of the intestine to the first-pass effect is more prominent; the converse also holds true. 4MUG, a dominant metabolite of the intestine, was reduced with increased flow, whereas 4MUS, a dominant metabolite of the liver, became more prominent in the overall first-pass metabolism. The concepts developed herein will apply to first-pass drug metabolism *in vivo*. In *in vivo*, flow to the intestine is known to increase with hypoxic vasodilation and gastrointestinal hormones such as secretin, neurotensin and cholecystokinin (Chou *et al*., 1984; Premen *et al*, 1985). Moreover, food intake (Svensson *et al*., 1983; Olanoff *et al*., 1986; Moneta *et al*., 1988) and vasoactive agents (Svensson *et al*., 1985) are known to increase intestinal and liver blood flow. Stimulation by glucose in lumen (Shepherd and Granger, 1984) can further increase intestine blood flow, and the increase is mainly to the mucosal layer (Granger *et al*., 1980; Yu *et al*., 1975; Shepherd and Riedel, 1985). Flow rate to the intestine is decreased in elderly sick patients, congestive

cardiac failure, digitalis toxemia, arteriosclerosis and intestinal ischemia (Jacobson, 1991). These flow changes to the intestine and liver are expected to bring about altered contributions of intestinal *versus* hepatic metabolism for drugs that are metabolized by both the intestine and liver.

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References

Ahmad, A., Bennett, P. N. and Rowland, M.: Models of hepatic drug clearances: discrimination between the "well-stirred" and "parallel tube" models. J. Pharm. Pharmacol. **35:** 219–224, 1983.

Brauer, R. W., Leong, G. F., McElroy, R. F. and Holloway, R. J.: Hemodynamics of the vascular tree of the isolated rat liver preparation. Am. J. Physiol. **186:** 537–542, 1956.

Chou, C. C., Mangino, M. J. and Sawmiller, D. R.: Gastrointestinal hormones and intestinal blood flow. *In* Physiology of the Intestinal Circulation, ed. by A. P. Shepherd and D. N. Granger, pp. 121–130, Raven Press, New York, 1984.

Dawson, J. R., Weitering, J. G., Mulder, G. J., Stillwell, R. N. and Pang, K. S.: Alteration of transit time and direction of flow to probe the heterogeneous distribution of conjugating activities for harmol in the perfused rat liver preparation. J. Pharmacol. Exp. Ther. **234:** 691–697, 1985.

DeLannoy, I. A. M. and Pang, K. S.: Combined recirculation of the rat liver and kidney: Studies with enalapril and enalaprilat. J. Pharmacokinet. Biopharm. **21:** 423–456, 1993.

El-Mouelhi, M. and Kauffman, F. C.: Sublobular distribution of transferases and hydrolases associated with glucuronide, sulfate and glutathione conjugation in human liver. Hepatology **6:** 450–456, 1986.

Granger, D. N., Richardson, P. D. I., Kvietys, P. R. and Mortillaro, N. A.: Intestinal blood flow. Gastroenterology **78:** 837–863, 1980.

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

Hirayama, H., Xu, X. and Pang, K. S.: Viability of the vascularly perfused, recirculating rat intestine and intestine-liver preparations. Am. J. Physiol. **257:** G249–G258, 1989.

Jacobson, E. D.: The splanchnic circulation. *In* Gastrointestinal Physiology, 4th ed., , ed. by L. R. Johnson, pp. 142–161, St. Louis, Mosby-Year Book, 1991.

Lindros, K. O.: The once-through perfusion technique-advantages and limitations. *In* Regulation of Hepatic Metabolism: Alfred Benzoin Symposium VI, ed. by F. Lundqvist and N. Tygstrup, pp. 778–789, Academic Press, Karger, Basal, 1974.

Moneta, G. L., Taylor, D. C., Helton, W. S., Mulholland, M. W. and Strandness, Jr., D. E.: Duplex ultrasound measurement of postprandial intestinal blood flow: Effect of meal composition. Gastroenterology **95:** 1294–1301, 1988.

Morris, M. E. and Pang, K. S.: Competition between two enzymes for substrate removal in liver: Modulating effects of competitive pathways. J. Pharmacokinet. Biopharm. **15:** 473–496, 1987.

Morris, M. E., Yuen, V., Tang, B. K. and Pang, K. S.: Competing pathways in drug metabolism. I. Effect of input concentration on the conjugation of gentisamide in the once-through in situ perfused rat liver preparation. J. Pharmacol. Exp. Ther. **245:** 614–622, 1988.

Mulder, G. J., Brouwer, S. and Scholtens, E.: High-rate conjugation of 4-methylumbelliferone during intravenous infusion in the rat *in vivo*. Biochem. Pharmacol. **33:** 2341–2344, 1984.

Mulder, G. J., Brouwer, S., Weitering, J. G., Scholtens, E. and Pang, K. S.: Glucuronidation and sulfation in the rat *in vivo*. The role of the liver and the intestine in the in vivo clearance of 4-methylumbelliferone. Biochem. Pharmacol. **34:** 1325–1329, 1985.

Olanoff, L. S., Walle, T., Cowart, T. D., Walle, U. K., Oxemann, M. J. and Conradi, E. C.: Food effects on propranolol systemic and oral clearance: Support of a blood flow hypothesis. Clin. Pharmacol. Ther. **40:** 408–414, 1986.

Pang, K. S., Koster, H., Halsema, I. C. M., Scholtens, E. and Mulder, G. J.: Aberrant pharmacokinetics of harmol in the perfused rat liver preparation: Sulfate and glucuronide conjugations. J. Pharmacol. Exp. Ther. **219:** 134–140, 1981.

Pang, K. S., Koster, H., Halsema, I. C. M., Scholtens, E., Mulder, G. J. and Stillwell, R. N.: Normal and retrograde perfusion to probe the zonal distribution of sulfation and glucuronidation activities of harmol in the perfused rat liver preparation. J. Pharmacol. Exp. Ther. **224:** 647–653, 1983.

Pang, K. S., Lee, W. F., Cherry, W. F., Yuen, V., Accaputo, J., Fayz, S., Schwab, A. J. and Goresky, C. A.: Effects of perfusate flow rate on measured blood volume, Disse space, intracellular water space, and drug extraction in the perfused rat liver preparation: Characterization by the multiple indicator dilution technique. J. Pharmacokinet. Biopharm. **16:** 595–632, 1988.

Pang, K. S. and Mulder, G. J.: A commentary: The effect of flow on formation of metabolites. Drug Metab. Dispos. **18:** 270–275, 1990.

Pang, K. S. and Rowland, M.: Hepatic clearance of drugs. I. Theoretical considerations of a "well-stirred" model and a "parallel tube" model. Influence of hepatic blood flow, plasma and blood cells binding, and the hepatocellular enzymatic activity on hepatic drug clearance. J. Pharmacokinet. Biopharm. **5:** 625–653, 1977a.

Pang, K. S. and Rowland, M.: Hepatic clearance of drugs. II. Experimental evidence for acceptance of the "well-stirred" model over the "parallel tube" model using lidocaine in the perfused rat liver in situ preparation. J. Pharmacokinet. Biopharm. **5:** 655–680, 1977b.

Pang, K. S. and Rowland, M.: Hepatic clearance of drugs. III. Additional experimental evidence supporting the "well-stirred" model using metabolite (MEGX) data generated from lidocaine under varying hepatic blood flow rate and linear conditions in the perfused rat liver in situ preparation. J. Pharmacokinet. Biopharm. **5:** 681–690, 1977c.

Premen, A. J., Kvietys, P. R. and Granger, D. N.: Postprandial regulation of intestinal blood flow: role of gastrointestinal hormones. Am. J. Physiol. **249:** G250–G255, 1985.

Ratna, S., Chiba, M., Bandyopadhyay, L. and Pang, K. S.: Futile cycling between 4-methylumbelliferone and its conjugates in perfused rat liver. Hepatology **17:** 838–853, 1993.

Severinghaus, J. W.: Oxyhemoglobin dissociation curve correction for temperature and pH variation in human blood. J. Appl. Physiol. **12:** 485–486, 1958.

Shand, D. G., Kornhauser, D. M. and Wilkinson, G. R.: Effects of route of administration and blood flow on hepatic elimination. J. Pharmacol. Exp. Ther. **195:** 424–432, 1975.

Shephard, A. P. and Granger, D. N.: Metabolic regulation of the intestinal circulation. **In** Physiology of The Intestinal Circulation, ed. by A. P. Shepherd and D. N. Granger, pp. 33–47, Raven Press, New York, 1984.

Shepherd, A. P. and Riedel, G. L.: Laser-Doppler blood flowmetry of intestinal mucosal hyperemia induced by glucose and bile. Am. J. Physiol. **248:** G393–G397, 1985.

St-Pierre, M. V., Schwab, A. J., Goresky, C. A., Lee, W.-F. and Pang, K. S.: The multiple indicator dilution technique for characterization of normal and retrograde perfusions in the once-through rat liver preparation. Hepatology **9:** 285–296, 1989.

Svensson, C. K., Cumella, J. C., Tronolone, M., Middleton, E. and Lalka, D.: Effects of hydralazine, nitroglycerin, and food on estimated hepatic blood flow. Clin. Pharmacol. Ther. **37:** 464–468, 1985.

Svensson, C. K., Edwards, D. J., Mauriello, P. M., Barde, S. H., Foster, A. C., Lanc, R. A., Middleton, E. and Lalka, D.: Effect of food on hepatic blood flow: Implications in the "food effect" phenomenon. Clin. Pharmacol. Ther. **34:** 316–323, 1983.

Whitsett, J. L., Dayton, P. G. and Mcnay, T. L.: The effect of hepatic blood flow on the hepatic removal rate of oxyphenbutazone in the dog. J. Pharmacol. Exp. Ther. **177:** 246–255, 1971.

Wilkinson, G. R.: Clearance approaches in pharmacology. Pharmacol. Rev. **39:** 1–47, 1987.

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

Xu, X., Tang, B. K. and Pang, K. S.: Sequential metabolism of salicylamide exclusively to gentisamide-5-glucuronide and not gentisamide sulfate conjugates in the single pass *in situ* perfused rat liver. J. Pharmacol. Exp. Ther. **253:** 963–973, 1990.

Yu, Y. M., Yu, L. C. C. and Chou, C. C.: Distribution of blood flow in the intestine with hypertonic glucose in the lumen. Surgery **78:** 520–525, 1975.

Zimmerman, C. L., Ratna, S., Leboeuf, E. and Pang, K. S.: High-performance liquid chromatographic method for the direct determination of 4-methylumbelliferone and its glucuronide and sulfate conjugates. J. Chromatogr. **563:** 83–94, 1991.

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