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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[1]

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Theoretical analysis of two models of hepatic drug clearance revealed that one powerful discriminator between them is the effect of changes of hepatic blood flow on either the emergent drug concentration or the availability of a highly extracted compound when operating under linear conditions. Lidocaine (extraction ratio 0.997) was employed in the discriminatory studies. The behavior of this drug under linear conditions (input lidocaine concentrations <5 mg/liter) to changes in hepatic blood flow rate (10–16 ml/min per liver) was examined in the perfused rat liver in situ *preparation. The steady-state output lidocaine concentration in the blood leaving the liver was predicted better by a "well-stirred" model than by a "parallel tube" model. As anticipated, the clearance of a poorly extracted compound, antipyrine (extraction ratio 0.08), was unaltered by changes in hepatic blood flow. These experimental findings, and the data from the literature, point to the acceptance of the "well-stirred" model, which describes the liver as a well-stirred compartment with the drug in the hepatic venous blood being in equilibrium with that in the liver.*

KEY WORDS: hepatic drug clearance; "well-stirred" model; "parallel tube" model; discrimination; steady-state output concentration; lidocaine; antipyrine.

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INTRODUCTION

The importance of the liver as an eliminating organ for drugs is well recognized. Its unique anatomical position with regard to the elimination of orally administered drugs poses many problems. The manner in which alterations in physiological states, such as organ blood flow, degree of drug binding within blood, and hepatocellular enzymatic activity, influence the hepatic handling of drugs and hence their oral availability is poorly understood.

The properties of two commonly used models of hepatic drug clearance operating under steady-state conditions were examined theoretically in our earlier publication (1). The "well-stirred" model (model I) (2) assumes that the liver behaves like a well-stirred tank, with drug in the blood emerging from the liver in equilibrium with that in the liver. The "parallel tube" model (model II) (3,4) assumes that the liver consists of identical and parallel tubes, with enzymes distributed evenly within the cells lining these tubes. The two models differ in their predictions of hepatic clearance, and thus in predicting other pharmacokinetic parameters as well (1). The choice of the model can lead to different interpretations of the data. Therefore, there is a need to discriminate between these two models, and to determine which, if either, should be used to predict the properties of the liver as an organ of elimination.

Theoretical considerations from our previous publication (1) have shown that the discrimination between the models is best performed under steady-state and linear conditions, where all pharmacokinetic parameters are concentration independent. The suitability of a pharmacokinetic parameter to discriminate between model I and model II is assessed by the degree of discrepancy that exists between its values predicted by these models. The analysis (1) showed that the following parameters—availability, steady-state drug concentration in hepatic venous blood, area under the blood drug concentration–time curve following a single oral dose, and steady-state drug concentration in blood following constant oral drug administration—are all excellent discriminators when the behavior of a highly extracted drug is examined under perturbations of either flow or of degree of binding within blood. However, no parameter of a poorly extracted compound can be used to discriminate between the models of hepatic clearance.

EXPERIMENTAL

The perfused rat liver *in situ* preparation was used. The perfusion apparatus (Fig. 1) and the perfusion technique were similar to those described by Mortimore (5). The differences were that a peristaltic pump

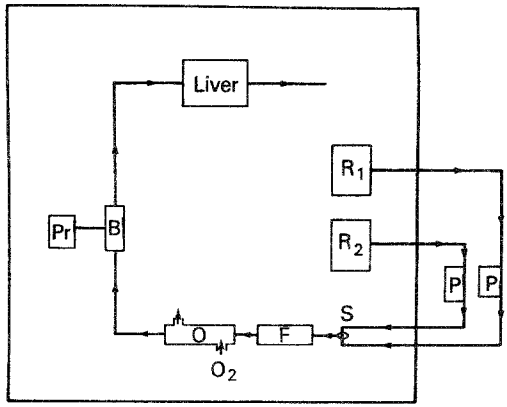

Fig. 1. Schematic representation of the assembly of the perfusion apparatus. R_1 and R_2 are rotating reservoirs 1 and 2. P, S, O, F, B, Pr, and O_2 represent the Harvard peristaltic pump, the four-way stopcock, the hollow fiber oxygenator, the blood filter, the bubble trap, the manometer, and the carbogen, respectively. The big square delineates the boundary of the perfusion chamber where the temperature is maintained at 37°C. The arrows indicate direction of flow.

(Harvard Apparatus Co., Millis, Maine) was used instead of a diaphragm pump; a hollow fiber oxygenator (Edwards Laboratories, Santa Ana, California) was used instead of the passing of oxygen directly over the rotating reservoir of blood; and the Bentley partial bypass filter (Coast Medical Corp., Walnut Creek, California), a sphygmomanometer (American Hospital Supplies, South San Francisco, California), and a bubble trap were incorporated into the system.

Male Sprague Dawley rats (350–400 g) were used. The surgical procedure was as follows. After the animals had been anesthetized with intraperitoneal injection of pentobarbital (Abbott Labs, Chicago, Illinois, 50 mg/kg), the bile duct was first cannulated with PE 20 tubing (Clay Adams, Parisippany, New Jersey) followed by cannulation of the portal vein (Cathlon IV catheter placement unit 16G-$2\frac{1}{2}''$, Jelco, Raritan, New Jersey). Perfusate medium containing 20% washed-out, dated human red blood cells, 1% bovine serum albumin (Sigma, St. Louis, Missouri), 3% dextran (Pharmacia, Piscataway, New Jersey), 300 mg% glucose (Travenol Labs, Deerfield, Illinois) in Krebs–Ringer bicarbonate solution (6) buffered to *p*H 7.4 and equilibrated with 95% oxygen and 5% carbon dioxide (Carbogen, Ohio Medical Products, Madison, Wisconsin) was immediately delivered to the liver. The outflow from the liver was collected by means of

a second catheter (Cathlon IV catheter placement unit 14G-2½", Jelco, Raritan) inserted through the right atrium into the inferior vena cava. The liver was perfused *in situ* and the animal was lowered into the perfusion chamber with the temperature maintained at 37 ± 1°C. Perfusion studies lasted between 2–3 hr.

Viability Tests

The "plasma" portion of the perfusate (5 ml) was assayed (ICN Laboratories, Portland, Oregon) for glucose, uric acid, creatinine, BUN, LDH, SGOT, SGPT, bilirubin, alkaline phosphatase, and electrolytes (phosphorus, chloride, sodium, potassium, and calcium). Oxygen tension, *p*H, and P_{CO_2} from both the arterial (input) and the venous (output) blood were measured (Radiometer, Copenhagen). Perfusion pressure was monitored by a Tyco aneroid sphygmomanometer that was connected to the apparatus via a bubble trap (Fig. 1). Electron microscopy was performed on a liver that was randomly selected after cessation of the experiment.

Sampling

For steady-state experiments (single pass without recirculation of medium), blood sampling from the reservoir was performed by taking appropriate aliquots at zero time (before commencement of perfusion studies) and at the end of the perfusion period for each set of steady-state perfusion periods. The input drug concentration (C_{In}) in the blood was taken as the mean of these determinations. The outflow blood was collected within the last 10 min of each steady-state period (25–30 min); the mean of the concentrations of drug in four consecutive 3-min samples was taken as the steady-state drug concentration in blood leaving the liver (C_{Out}). For the recirculating experiments, appropriate aliquots of the perfusate were taken from the reservoir at zero time and at subsequent time intervals. Bile was collected *in toto* in all experiments. The liver was removed rapidly from the carcass immediately after the perfusion studies, and was cut into fine pieces before it was immersed in ice-cold methanol for 30 min (1–3 min for operation). The liver was then weighed. After the addition of 10 N NaOH, the liver was homogenized (50-ml glass homogenizer). All blood, bile, and liver samples were stored in a refrigerator before analysis.

Assay Methods

Lidocaine (2-diethylamino-2',6'-acetoxylidide, Astra, Worchester, Massachusetts) was determined by a gas–liquid chromatographic procedure similar to that described by Benowitz and Rowland (7); the internal

standard used was W12714 (2-ethyl-2-isobutyl-2′,6′-acetoxylidide, Astra). Monoethylglycine xylidide, MEGX (2–ethylamino-2′,6′-acetoxylidide, Astra), a metabolite of lidocaine, was determined by gas–liquid chromatography (8). Antipyrine (Merck and Co., Inc., Rahway, New Jersey) was assayed by gas–liquid chromatography (9).

Protein Binding and Protein Determination

The degree of binding was determined over a wide concentration range of lidocaine by equilibrium dialysis (Dianorm, Scientific and Medical Instrumentation, CH-Esslinger, Switzerland) (10). One milliliter of perfusate was equilibrated against 1 ml Krebs–Ringer bicarbonate, both buffered to *p*H 7.4. The degree of binding of lidocaine to the "plasma" components of the perfusate was determined by spiking the "plasma" samples with 10 μl of an aqueous solution containing 0.17 μg ^{14}C-carbonyl lidocaine hydrochloride (specific activity 4.3 mCi/mmole, New England Nuclear, Boston, Massachusetts). Dialysis experiments were run for 3 hr; preliminary experiments indicated that equilibrium was achieved within $1\frac{1}{2}$ hr. After equilibration, a 200-μl aliquot was taken from the "plasma" and the buffer side, and the radioactivity was counted (Tricarb, Packard, Downers Grove, Illinois). Protein determination on the buffer side of the system was based on a modification of the Lowry method (11), and absorbance of the samples was read at 750 nm with a spectrophotometer (Cary 15, Varian Instruments Inc., Palo Alto, California).

EQUATIONS

The basic equations defining elimination across an organ at steady state are

$$\text{velocity, } v = Q(C_{\text{In}} - C_{\text{Out}}) \tag{1}$$

$$\text{extraction ratio, } E = 1 - C_{\text{In}}/C_{\text{Out}} \tag{2}$$

where Q is the organ blood flow, and C_{In} and C_{Out} are the concentrations of drug in blood entering and leaving the organ, respectively. The equations below have been derived in an earlier publication (1). For a unienzyme system, the velocity of the reaction at steady-state is defined for model I by

$$v = \frac{V_{\max} C_{\text{Out}}}{K_m/f_{B,\text{Out}} + C_{\text{Out}}} \tag{3}$$

and for model II by

$$v = \frac{V_{max}\hat{C}}{K_m/f_{B,Out} + \hat{C}} \tag{4}$$

where V_{max} is the maximum velocity, K_m is the Michaelis–Menten constant of the enzyme, and $f_{B,Out}$ is the ratio of the unbound drug concentration in plasma to the whole blood drug concentration in blood leaving the liver. The term $\hat{C}$, given by $(C_{In} - C_{Out})/\ln (C_{In}/C_{Out})$, is the logarithmic average of C_{In} and C_{Out}.

When operating under linear conditions, the extraction ratio is defined for model I by

$$E = f_{B,Out}CL_{int,l}/(f_{B,Out}CL_{int,l} + Q) \tag{5}$$

and for model II by

$$E = 1 - e^{-f_{B,Out}CL_{int,l}/Q} \tag{6}$$

where $CL_{int,l}$ is the intrinsic clearance of the liver to eliminate the drug when operating under linear conditions (1).

RESULTS AND DISCUSSION

The Perfused Rat Liver System

The perfused rat liver preparation is an ideal way to look at the underlying problem of discrimination between the two models of hepatic drug clearance. This *in vitro* preparation is close to the *in vivo* situation, because the liver can be maintained in a viable and stable state as oxygen and nutrients are being delivered adequately to the liver by the perfusate under physiological hepatic blood flow rates. The preparation also allows easy control over the experimental conditions, since the flow rate and the composition of the medium are easily manipulated. Recirculating and single-pass experiments can be designed; the latter is advantageous in that large samples of the emergent blood can be taken.

Chemical Analysis

Lidocaine Assay

The slopes of the calibration curves prepared from samples containing lidocaine hydrochloride monohydrate (0.0625–2 μg) and W12714 (1 μg) in blood, plasma, water (in 2 N NaOH) were not significantly different, at the 95% confidence level (Fig. 2). Furthermore, the slope of the calibration curve with the blood samples was found constant over days 1, 2, and 3

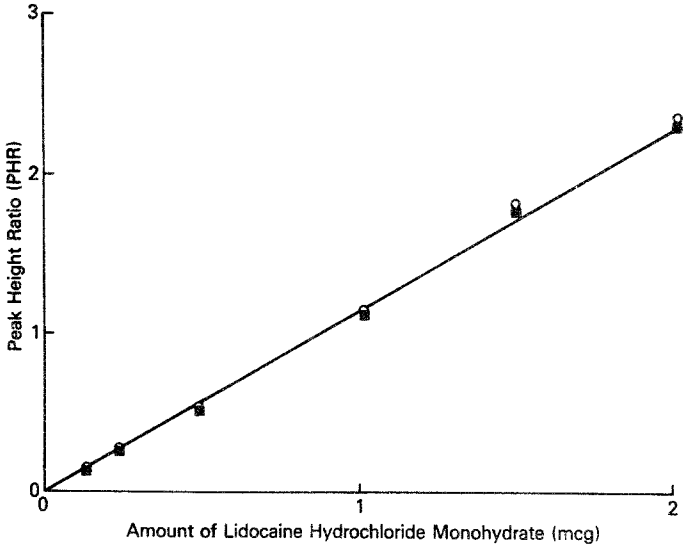

Fig. 2. Calibration curve for lidocaine. The peak height ratio of lidocaine to its internal standard is plotted against the amount of lidocaine hydrochloride monohydrate (0.125–2.0 μg) added to biological samples. ○, Calibration curve of samples in blood; ■, calibration curve of the samples in liver (in 10 N NaOH).

upon reinjection of the same samples, indicating that lidocaine is stable in its solvent, carbon disulfide, for at least 3 days after the extraction procedure. The stability of lidocaine in strong base (10 N NaOH) was tested by repeating the liver analysis 6 months subsequent to the initial analysis. When samples from six different livers were reexamined, the data (Table I) showed little difference in the lidocaine content in the livers, implying that lidocaine is stable in strong base. This supports the findings of Benowitz and Rowland (7). To assess the reproducibility of the assay, five blood samples containing 1 μg lidocaine hydrochloride monohydrate and 1 μg W12714 were taken through the assay procedure. The mean peak height ratio was 1.20, with a coefficient of variation of 1.7%. The reproducibility of the assay between and within days was assessed from the slopes of nine calibration curves from nine sets of analyses on different days. The mean of these nine slopes was 1.30, with a coefficient of variation of 6%.

Table I. Stability of Lidocaine in Liver Tissues Stored in 10 N Sodium Hydroxide

	Amount of lidocaine in liver tissues (μg)					
First analysis	27.1	15.0	31.4	39.1	23.8	68.9
Second analysis (6 months later)	28.5	21.5	28.8	39.5	25.3	72.9

Table II. Peak Height Ratio with Changes in the Volume Ratio of Blood to Ether for 0.5 μg Lidocaine Hydrochloride Monohydrate and 4.5 μg of Internal Standard

Volume of blood	Volume of ether	Volume ratio blood/ether	Peak height ratio
0.25	6	0.042	0.185
2.25	6	0.375	0.215
4.25	6	0.708	0.227
6.25	6	1.042	0.236
8.25	6	1.375	0.234
2	2	1.0	0.227
2	4	0.5	0.243
2	6	0.33	0.233
2	8	0.25	0.241
2	10	0.2	0.250

Large aliquots (up to 15 ml) were often used in the analysis of the output lidocaine concentrations, whereas small volumes of blood (0.25 ml) were used for analysis of the input concentrations. Although the volume ratio of blood to organic solvent was always adjusted to be the same with blank blood for each set of analyses, the degree of extraction of lidocaine and its internal standard into the organic phase (ether) was tested against different volume ratios of blood to ether. The data (Table II) showed no trend in the relative extraction of lidocaine to its internal standard over a tenfold change in the volume ratio. The specificity of the lidocaine assay was tested for possible interference by its metabolite, MEGX, by taking a mixture of 20 μg MEGX, 0.125 μg lidocaine hydrochloride monohydrate, and 1 μg W12714 in blood through the assay procedure. The peak height ratio of lidocaine to internal standard was not affected by the presence of a large amount of MEGX. The absence of a peak at the retention time of lidocaine when blank blood containing 1 μg W12714 was taken through the assay procedure indicated the lack of interference from constituents in blood. The minimum amount of lidocaine detected by the procedure was 50 ng.

Antipyrine Assay

The slopes of the calibration curves from samples containing antipyrine (0.625–10 μg) and its internal standard, 4-bromoantipyrine (10 μg), in blood, plasma, and water were not significantly different at the 95% confidence level (Fig. 3). The slope of the calibration curve from samples in blood, taken through the assay procedure, remained constant over days 1, 2, and 3 upon reinjection of the same samples, indicating that antipyrine is stable for at least 3 days after the extraction procedure. The stability of antipyrine in phosphate buffer (*p*H 7.4) was demonstrated by

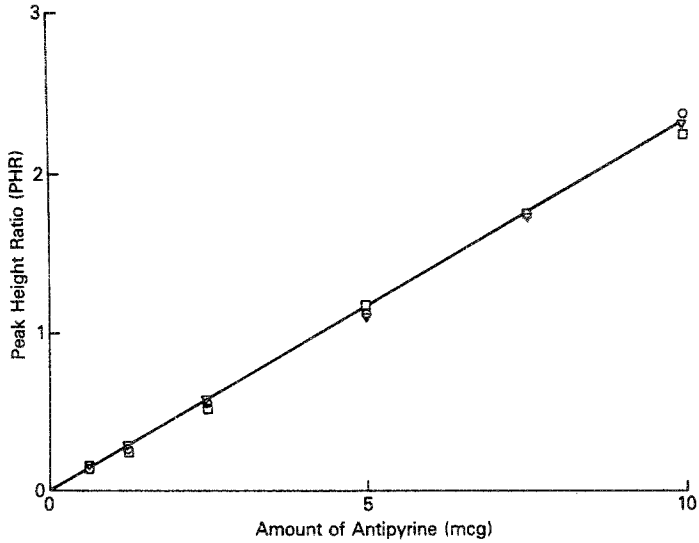

Fig. 3. Calibration curve for antipyrine. The peak height ratio of antipyrine to its internal standard is plotted against the amount of antipyrine in biological samples. ○, Calibration curve of antipyrine in blood; □, calibration curve in plasma; △, calibration curve in water.

the presence of reproducible slopes when the same stock solution was used as standard at different times. To assess the reproducibility of the assay, four blood samples containing 10 μg of each of antipyrine and 4-bromoantipyrine were taken through the assay procedure. The mean peak height ratio was 2.36, with a coefficient of variation of 2%. The reproducibility of the assay between and within days was assessed from the slopes of four calibration curves from four sets of analyses on separate days. The mean of these four slopes was found to be 2.3, with a coefficient of variation of 0.8%. The absence of a peak at the retention time of antipyrine when blank blood was assayed indicated the specificity of the procedure. The minimum amount of antipyrine detected was 125 ng.

MEGX Assay

The slopes of the calibration curves prepared from MEGX (50–750 ng) and its internal standard (500 ng) in blood and in plasma, taken through the assay procedure, were linear and did not differ from one another (slope 7.0) (Fig. 4). The slope of the calibration curve from samples in water, however, was 5.5. The cause of this discrepancy was not investigated further, but the calibration curve used in the perfusion studies was obtained by extracting known concentrations of MEGX from

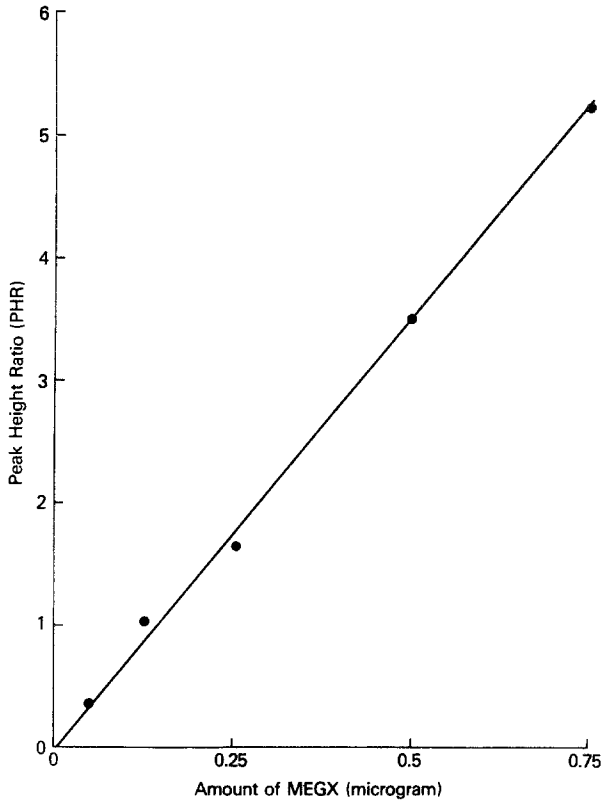

Fig. 4. Calibration curve for MEGX in blood. The peak height ratio of derivatized MEGX to its internal standard is plotted against the amount of MEGX (62.5–750 ng).

the perfusion medium for each set of analyses. The slope of the calibration curve for MEGX in blood was 6.5 for day 1, and upon reinjection of the same samples the slopes were linear but were 7.0 and 9.0, respectively. The change in the slope may be due to the deterioration of the derivatized internal standard upon standing. The stability of MEGX in phosphate buffer (*p*H 7.4) was shown by the reproducibility of the slopes when MEGX from the same stock solution was used at different times. To assess the reproducibility of the assay, six blood samples containing 125 ng MEGX and 500 ng of the internal standard were taken through the assay procedure. The mean peak height ratio was 1.70, with a coefficient of variation of 9%. Specificity of the MEGX assay was demonstrated by the absence of a change in the peak height ratio of the derivatized MEGX (25 ng) to that of the internal standard, before and after the addition of a

large amount of lidocaine (10 ng), and by the absence of interfering peaks at the retention time of MEGX when blank blood was taken through the assay procedure. The minimum amount of MEGX detected was 10 ng.

Protein Binding

The concentrations of lidocaine in blood and in plasma and the degree of binding of lidocaine to bovine serum albumin were determined simultaneously from the same sample of perfusate. The data (Table III) showed that lidocaine did not bind to the components in the perfusate; the ratio of the unbound plasma concentration to the whole blood drug concentration ($f_{B,Out}$) is equal to 1 (95% confidence interval). This finding is consistent with the views of Tucker *et al.* (12) that lidocaine does not bind significantly to the albumin fraction in human plasma. Antipyrine is known to distribute in total plasma water, and binds only negligibly to plasma proteins (13). The fraction of antipyrine unbound in the perfusate is thus taken as unity.

Table III. Binding of Lidocaine to Perfusate Components

Lidocaine concentration before equilibrium dialysis[a] (mg/liter)		Counts/200 μl after equilibrium dialysis[b]		Fraction unbound	
"Blood" C_B	"Plasma" C_P	Buffer side	"Plasma" side	"Plasma" f_P[c]	"Blood" f_B[d]
2.93	3.21	537	601	0.89	0.98
3.64	3.88	583	651	0.90	0.96
8.58	8.46	538	581	0.93	0.92
19.04	20.68	674	737	0.93	1.01
36.33	35.50	560	624	0.89	0.87
0.005	0.004	536	603	0.89	0.71
0.013	0.013	596	624	0.95	0.95
0.011	0.012	516	548	0.94	1.02
0.16	0.15	521	539	0.97	0.96
0.15	0.15	652	677	0.96	0.96
2.87	2.97	529	519	1.02	1.05
2.91	2.91	593	617	0.96	0.96
8.74	8.45	559	564	0.96	0.93
				Mean	0.95
				n	13
				SE	±0.023

[a] Assayed by the GLC method.
[b] Samples spiked with radioactivity, same quenching in all samples.
[c] Counts on buffer side/counts on "plasma" side.
[d] $f_P C_P / C_B$.

Viability and Steady State

Before any interpretations could be made, the viability and the stability of the preparation had to be established. The biochemistry of the medium, the oxygen tension, the perfusion pressure, as well as electron microscopy all indicated general overall integrity and viability of the liver preparation. The best test was that the hepatic extraction ratio of the drug remained constant with time. The attainment of this criterion is illustrated in Fig. 5, when a constant-input lidocaine concentration (3.53 mg/liter) was delivered at a constant flow rate of 10 ml/min/liver. The output lidocaine concentration was low (0.0115 mg/liter) and constant throughout the study, indicating that lidocaine is extremely well cleared by the rat liver (extraction ratio 0.997), and that the preparation remained stable and viable. Other experiments indicated viability up to at least 3 hr. Since the lidocaine output concentration remained unaltered from 7 min (the first sampling point, Fig. 5) onward, it is concluded that steady-state conditions are achieved within this short period. The bile concentration was low, and the total amount (<0.4% of dose) indicated that biliary excretion of lidocaine constitutes only a minor eliminating pathway. Furthermore, liver analysis of lidocaine (this study and subsequent studies) accounted for less than 0.5% of the dose given. Additionally, when ^{14}C-carbonyl lidocaine was tested in the perfusion apparatus in the presence and absence of a liver, it was shown that lidocaine was not absorbed by the tubing in the perfusion apparatus. All these observations confirmed that lidocaine is highly metabolized by the liver.

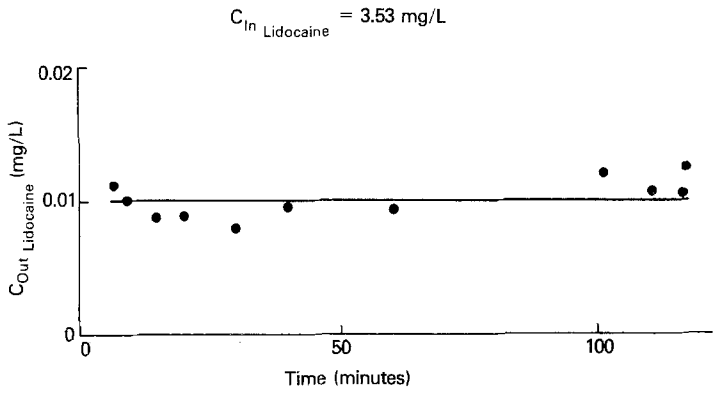

Fig. 5. Viability of the preparation as indicated by the constancy of the output lidocaine concentration with respect to time when a constant-input lidocaine concentration (3.53 mg/liter) was perfused under conditions of constant blood flow (10 ml/min per liver).

Linearity

As shown in the theoretical analysis (1), discrimination between the two models of hepatic drug clearance is simplified under steady-state and linear conditions. To test for linearity, the concentration of lidocaine in the input was increased stepwise at 30-min intervals from 0.95 to 7.0 mg/liter under constant hepatic blood flow (10 ml/min per liter). The stability of the system was checked by returning to the initial input condition at the end of the experiment. The data (Table IV) showed no significant trend in either the availability or the extraction ratio with varying-input lidocaine concentrations, indicating that the system is linear and stable at lidocaine input concentrations below 7 mg/liter. Subsequent experiments were mostly conducted using a lidocaine input concentration of approximately 4 mg/liter.

Discrimination Between the Models Using Lidocaine Under Linear Conditions by Varying Hepatic Blood Flow

Single-pass experiments were performed at constant-input lidocaine concentrations at around 4 mg/liter. Since hepatic blood flow is easily monitored and controlled in the preparation, the steady-state output concentration of this highly cleared compound, lidocaine, in these single-pass experiments was used as the sensitive index for the discrimination between the two models of hepatic drug clearance. Preliminary studies with lidocaine indicated that a low hepatic blood flow rates (4 ml/min per liver), the hepatic extraction ratio of lidocaine was reduced—an observation unexplained by either model. This paradox may be explained following the reasoning of Brauer *et al.* (14), who attributed similar observations with colloidal chromic phosphate to the collapse of the hepatic vascular bed at both low perfusion pressures and flow rates. This collapse would lend to a

Table IV. Constant Extraction of Lidocaine with Varying Input Concentrations

Lidocaine concentrations (mg/liter)			
Input C_{In}	Mean output C_{Out}	Availability $F = C_{Out}/C_{In}$	Extraction ratio $E = (C_{In} - C_{Out})/C_{In}$
7.0	0.043 ± 0.008	0.0061	0.9939
5.73	0.026 ± 0.003	0.0045	0.9955
3.0	0.015 ± 0.001	0.0050	0.9950
1.95	0.009 ± 0.001	0.0046	0.9954
0.95	0.004 ± 0.001	0.0042	0.9958
6.53	0.057 ± 0.008	0.0087	0.9913
	$n = 4$ (SE)		

reduction of the effective area of the hepatic vasculature, resulting in a reduced extraction of the compound. At high perfusion flow rates (20 ml/min per liver), the liver suffered degenerative changes probably caused by excessive perfusion pressures and flows in the system. Thus hepatic blood flow was restricted to vary between 10 and 16 ml/min per liver.

The following design was adopted for the discriminatory studies. A control flow (e.g., 12 ml/min per liver) was chosen to perfuse the rat liver at a constant-input lidocaine concentration. After 25 min of perfusion, the flow rate was randomly changed to either 10, 14, or 16 ml/min per liver for 25 min before returning the flow back to the control value. This process was repeated for two additional flow changes. This scheme is illustrated in Fig. 6. The design provides a meaningful interpretation of the data, as the viability of the preparation does fluctuate with time. Since the flow change is imposed between the control flow periods, it is not unreasonable to consider the enzymatic activity of the system during the flow change period to be the average of that of the control flow periods. By so assuming, the effects of flow changes on the hepatic extraction of a compound are easily interpreted and interrelated, even when slight fluctuations exist in the stability of the preparation. From the input drug concentration and the mean of the output lidocaine concentrations at the control flow rate, the extraction ratio was calculated (*cf.* equation 2). By appropriate substitution of extraction ratio and blood flow into equations 5 and 6 (with $f_{B,Out} = 1$), the values of the intrinsic clearance according to models I and II, respectively, were calculated. Assuming that the intrinsic clearance is constant, a new extraction ratio and the steady-state output lidocaine concentration were then predicted for a new flow rate.

An example of a typical experiment is shown in Fig. 7, where the control flow rate was 10 ml/min per liver. The predicted values from

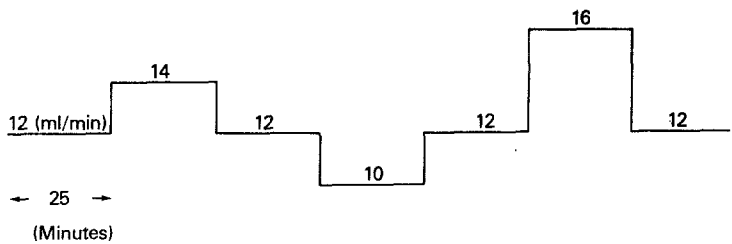

Fig. 6. Schematic representation of the design of the experiments with lidocaine used to discriminate between two models of hepatic drug clearance under linear kinetic conditions by varying hepatic blood flow rates. Each period of perfusion (25 min) is for the attainment of steady-state conditions. In this illustration, the control flow rate of 12 ml/min per liver is chosen and interrupted at random by flow rates of 10, 14, and 16 ml/min per liver.

models I and II were then compared against the observed data. From Fig. 7, it is seen that the predictions of model I fitted the data better than those of model II. An additional eight experiments were performed in the same manner, using 12 ml/min per liver as the control flow rate. The cumulative observations from the nine experiments were matched against their predictions for model I (Fig. 8) and for model II (Fig. 9). The straight line with the slope of 1 represents a perfect correlation between predicted and observed data. The observations were regressed against the predictions from the two models; the resultant slopes were compared to there straight line with the slope of 1. The *t* statistic for the comparison of slopes was not significant for model I (1.4008), but it was significant for model II (9.9649) at the 95% confidence level. The sum of squares of residuals was smaller for model I (0.00138) than for model II (0.0307). In addition to these statistical findings, pictorially, the degree of scatter of the data from the perfect correlation straight line (compare Figs. 8 and 9) suggests that model I better predicts the data.

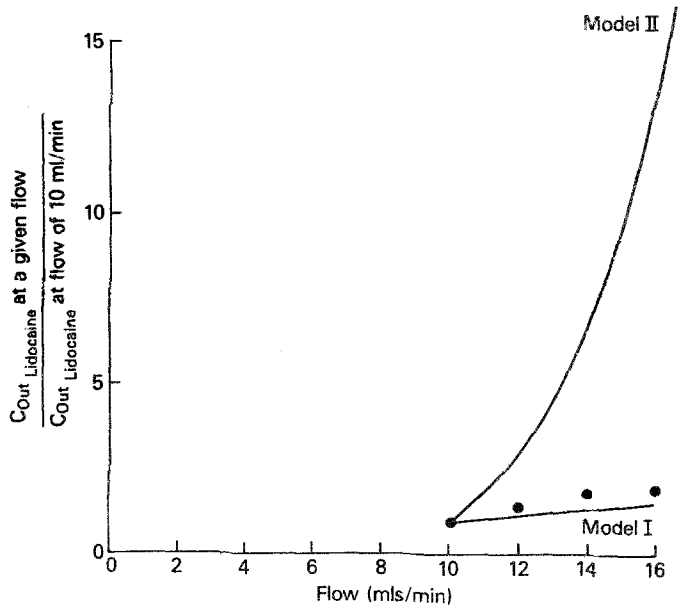

Fig. 7. Plot of the predicted and the observed lidocaine concentrations in the effluent blood for models I and II when hepatic blood flow was changed from the control flow rate of 10 to 12, 14, and 16 ml/min per liver. The lines represent predicted data from models I and II, and the points represent observations.

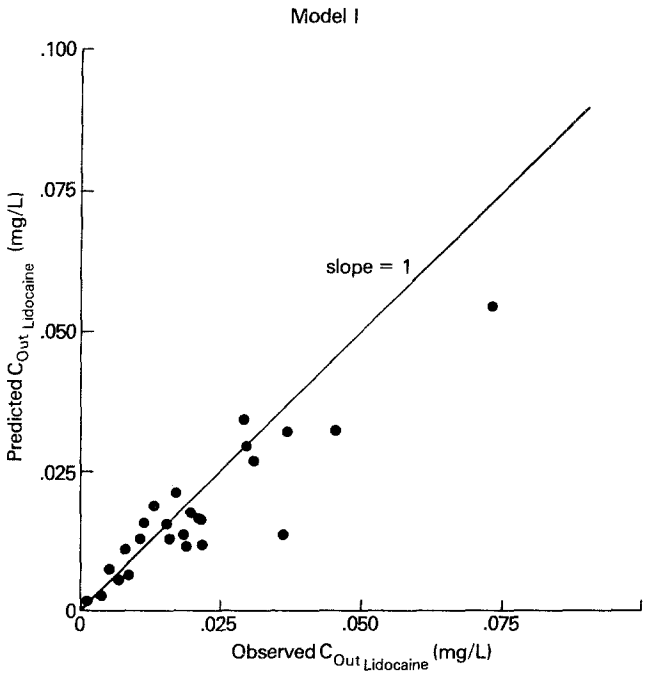

Fig. 8. Predicted vs. observed steady-state output lidocaine concentrations in the effluent blood from the liver for model I for nine experiments performed at low-input lidocaine concentrations (4 mg/liter) with alterations of hepatic blood flow. The straight line with the slope of 1 represents a perfect correlation between the predicted and observed data.

Hepatic Clearance of a Poorly Extracted Compound, Antipyrine, Under Varying Hepatic Blood Flow

Theoretical analysis (1) indicated that the clearance of a poorly extracted compound was insensitive to changes to hepatic blood flow, and hence such a compound is therefore a poor discriminator between various models of hepatic drug clearance. The insensitivity of a poorly extracted compound to changes in hepatic blood flow was tested using antipyrine, as preliminary single-pass perfusion studies indicated that the extraction ratio of this compound was low (0.08) in the perfused rat liver system. The differences between the input and output antipyrine concentrations were so small that they lay within the variability of the assay procedure. To circumvent this problem, recirculation of the perfusion medium was adopted. In this system, a bolus of drug was added initially to the reservoir, from which samples were taken subsequently. As predicted, the clearance of antipyrine remained unperturbed, as reflected by the lack of change in the

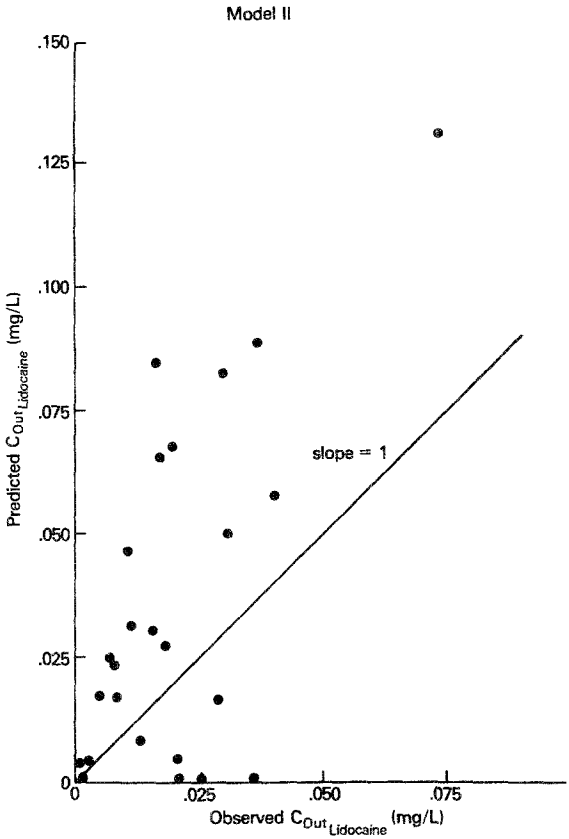

Fig. 9. The same observations from the nine experiments depicted in Fig. 8 were plotted against the predictions from model II. The straight line with the slope of 1 represents a perfect correlation between the predicted and observed data.

blood decay curve (Figs. 10 and 11) with changes in organ blood flow. It follows for such a drug that the extraction ratio changes inversely with hepatic blood flow (Table V).

Discrimination Between the Models Under Nonlinear Conditions with Lidocaine

Discrimination between the models was also attempted under nonlinear conditions, where the input lidocaine concentration was sufficiently high that the concentration of drug at the enzymatic site exceeded the concentration at half-maximum velocity, K_m, of the system. To examine the possibility of capacity limiting the enzyme system, varying concentrations

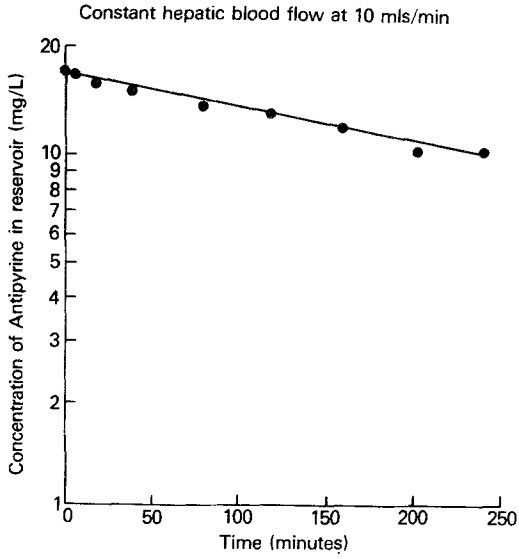

Fig. 10. Exponential decay of the concentrations of antipyrine in the reservoir with time in a recirculating experiment when the hepatic blood flow to the liver was kept constant at 10 ml/min per liver.

of lidocaine (3–84 mg/liter) were delivered at a constant hepatic blood flow (10 ml/min per liver). The concentration of lidocaine in the input was increased stepwise at 25- to 30-min intervals. The viability of the preparation was checked by repeating the initial condition. In a second study, the concentration of lidocaine was decreased stepwise. Table VI summarizes the findings and demonstrates concentration-dependent kinetics for lidocaine, with decreasing extraction ratios upon increasing the input lidocaine concentration.

Before analyzing the data, the possibility of end-product inhibition (15,16) was explored; as at high-input lidocaine concentrations, high-output metabolite (MEGX) concentrations were observed (0.25–30 mg/liter; MEGX constituted 8–55% of the input lidocaine concentration) (Table VI). Three studies were performed under conditions of constant hepatic blood flow (10 ml/min per liver) and low-input lidocaine concentrations (less than 3.5 mg/liter) with the metabolite MEGX added stepwise (0–13 mg/liter) to the input in each steady-state condition (25 min). In all cases, there was evidence of inhibition of the metabolism of lidocaine by MEGX (Table VII), although there was considerable variation between these studies.

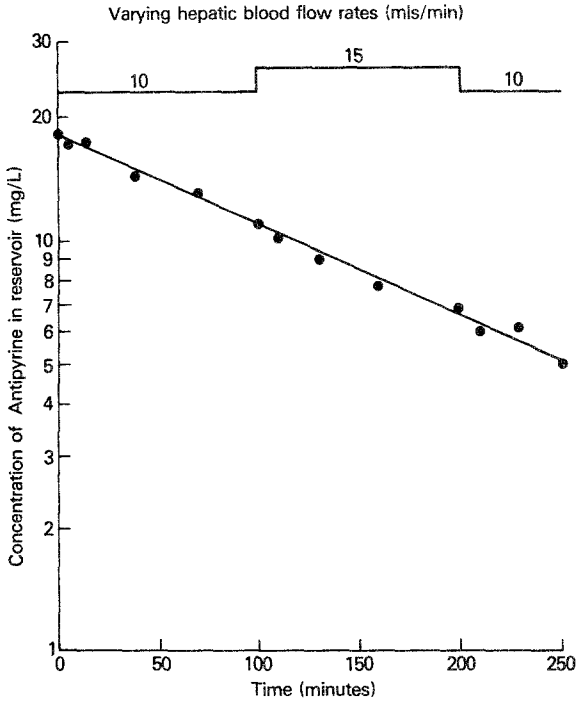

Fig. 11. Lack of effect of hepatic blood flow on the exponential decay of antipyrine concentrations in the reservoir with time in a recirculating experiment. Hepatic blood flow was interrupted from 10 to 15 ml/min per liver.

Table V. Various Parameters Calculated for Antipyrine in Recirculating Experiments

Study No.	Dose (μg)	Volume of reservoir (ml) V_R	Blood flow (ml/min per liver) Q	Half-life (min) $t_{1/2}$	$\bar{C}_B(0)^a$ (mg/liter)	Volume of distribution[b] (ml) V_D	Clearance[c] (ml/min) CL	Extraction ratio E
I	2777	160	10	145	17.2	161	0.77	0.077
II	3190	180	10	280	16.5	193	0.47	0.047
III	2742	150	10					0.078
			15	136	18.0	152	0.77	0.057
			10					0.078
IV	2707	140	16					0.036
			8	178	18.0	150	0.59	0.073
			16					0.036

[a] Extrapolated concentration at zero time.
[b] $V_D = \text{dose}/\bar{C}_B(0)$.
[c] $\text{CL} = 0.693\, V_D/t_{1/2}$.
[d] $E = \text{CL}/Q$.

Table VI. Effect of Lidocaine Concentration on Its Hepatic Extraction and Generation of Metabolite (MEGX) Under Constant Hepatic Blood Flow (10 ml/min per liver)

Study No.	Lidocaine concentration (mg/liter) Input C_{In}	Mean output C_{Out}	Extraction ratio of lidocaine E	Velocity of removal (μg/min) v	Output MEGX concentration (mg/liter)	MEGX as % of input lidocaine concentration[a]
I	2.9	0.004±0.001	0.9986	28.9	Not detected	—
	3.6	0.012±0.001	0.9967	36.1	0.25±0.03	7.9
	8.5	0.163±0.014	0.9812	83.4	1.34±0.08	17.9
	19.0	2.88 ±0.04	0.8526	161.2	6.50±0.2	38.8
	36.3	9.02 ±1.51	0.7515	272.8	12.2 ±0.3	38.2
	3.6	0.27 ±0.11	0.9250	27.3	0.96±0.2	30.3
II	84.3	20.4 ±0.7	0.7580	639.0	29.6 ±1.1	39.9
	38.6	9.3 ±0.39	0.7591	293.0	14.8 ±2.1	43.5
	19.1	3.7 ±0.39	0.8063	154.0	9.2 ±1.4	54.7
	9.1	1.4 ±0.27	0.8440	81.0	4.0 ±0.4	49.9
	4.4	0.21 ±0.06	0.9522	41.9	0.8 ±0.17	20.6
	39.9	7.4 ±0.9	0.8145	325.0	13.4 ±1.7	38.1
		$n=4$ (SE)			$n=4$ (SE)	

[a] Corrected for molecular weight.

Table VII. Inhibition of Metabolism of Lidocaine by MEGX

Study No.	Input concentration (mg/liter) Lidocaine	MEGX	Output concentration (mg/liter) Lidocaine	MEGX	Extraction ratio of lidocaine
I	3.0	0	0.006±0.001	0.027±0.003	0.9980
	3.0	0.76	0.028±0.009	0.170±0.015	0.9907
	3.0	2.4	0.11 ±0.025	0.629±0.058	0.9633
	3.0	5.3	0.64 ±0.027	2.06 ±0.077	0.7867
	3.0	11.3	2.7 ±0.11	6.65 ±0.45	0.1000
	3.0	0	0.006±0.001	0.073±0.033	0.9980
I	3.4	0	0.032±0.012	0.071±0.012	0.9906
	3.4	0.68	0.10 ±0.010	0.19 ±0.01	0.9706
	3.4	2.42	0.373±0.074	0.928±0.10	0.8903
	3.4	5.49	0.93 ±0.101	2.43 ±0.19	0.7265
	3.4	11.24	2.9 ±0.397	5.72 ±0.55	0.1471
	3.4	0	0.134±0.029	0.201±0.035	0.9606
III	3.3	0	0.004±0.0003	0.17 ±0.02	0.9988
	3.3	6.44	0.021±0.005	3.73 ±0.20	0.9936
	3.3	12.0	0.04 ±0.004	6.12 ±0.70	0.9879
	3.3	12.2	0.074±0.015	9.50 ±2.14	0.9776
	3.3	13.4	0.074±0.003	13.1 ±0.9	0.9776
	3.3	0	0.003±0.0003	0.18 ±0.14	0.9991
			$n=4$ (SE)	$n=4$ (SE)	

The situation may be further complicated by the possibility that elimination of lidocaine in the rat is mediated through a multienzyme system (17). A unienzyme system can be characterized reasonably by measuring only the rate of drug elimination and not necessarily the rate of formation of the products with changes in drug concentration. The adequacy of models I and II can then be assessed simply by fitting the observations to equations 3 and 4, although the discrimination between the models is difficult (1). For a multienzyme system, with increasing drug concentrations some metabolic pathways may become saturated while others remain linear. Such a system can be adequately characterized only by measuring both the elimination of the drug and the formation of all products. Moreover, the equations describing the drop in drug concentration across the liver for models I and II become more complex, and the fitting of the observations to the models becomes correspondingly more difficult. Because of the complexities mentioned above, no attempt was made to utilize the lidocaine data, under nonlinear conditions, to discriminate between the two models.

Literature Data

Data available in the literature regarding hepatic drug clearance and hepatic blood flow are scanty. Brauer *et al.* (18) determined the steady-state extraction ratio of radiocolloid chromic phosphate over a wide range of hepatic perfusion rates (0.5–6.0 ml/min per gram of liver) in the isolated perfused rat liver preparation. Whitsett *et al.* (19) reported the effects of changes in hepatic blood flow (0.46–2.0 ml/min/g liver) on the hepatic removal of oxyphenbutazone, a drug which is mostly cleared by the liver, in the intact dog. We fitted the data from both sources (extraction ratio vs. perfusion flow rate), using the nonlinear least-squares regression program NONLIN (20), to both models of hepatic drug clearance (Fig. 12A,B). The statistical parameters generated showed no significant difference between the models. Residual plots (Fig. 13) constructed from the data exhibited a fairly random distribution of the weighted residuals against the predicted value. Thus, using any statistical criteria (21), it is impossible to distinguish between the models based on these observations. This analysis confirmed our previous view that the extraction ratio is a poor discriminator between the models (1).

Branch *et al.* (22) related the extraction ratio of propranolol with hepatic blood flow in the perfused rat liver preparation. Their data were fitted equally well by the predictions of model I and model II. Subsequently, these workers (23) furnished more discriminatory evidence on the selection of the model; the steady-state lidocaine concentration in the systematic circulation after constant oral administration ($\bar{C}_{B,ss,oral}$)

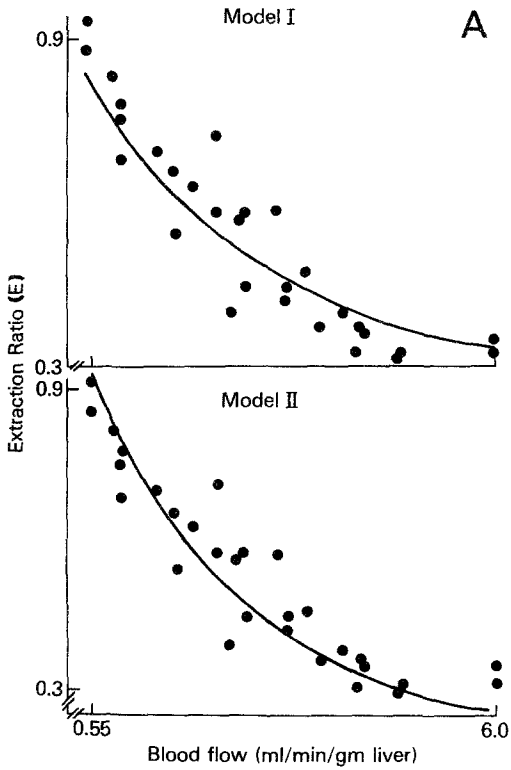

Fig. 12. NONLIN computer curve fitting of the data from (A) Brauer *et al.* (18) and (B) Whitsett *et al.* (19) for models I and II. The lines represent computer-generated lines, and the dots are experimental points.

remained unaltered when the hepatic blood flow was varied twofold. This latter finding is in accordance with the predictions of model I (1). In model II, for a highly cleared compound such as lidocaine, $\bar{C}_{B,ss,oral}$ is expected to change drastically with changes in hepatic blood flow. In the same publication (23), additional discriminatory evidence between the two models involving propranolol and diphenylhydantoin was also provided.

Keiding *et al.* (24) studied the elimination kinetics of galactose in a recirculating isolated perfused pig liver preparation under varying-input galactose concentrations, achieved by a stepwise increasing galactose infusion rate into the reservoir. Measurements were made of hepatic blood flow and of the concentration of substrate in the reservoir and in the blood entering and leaving the liver. Saturable elimination kinetics was observed. As galactose is metabolized by a single enzyme, galactokinase, the data

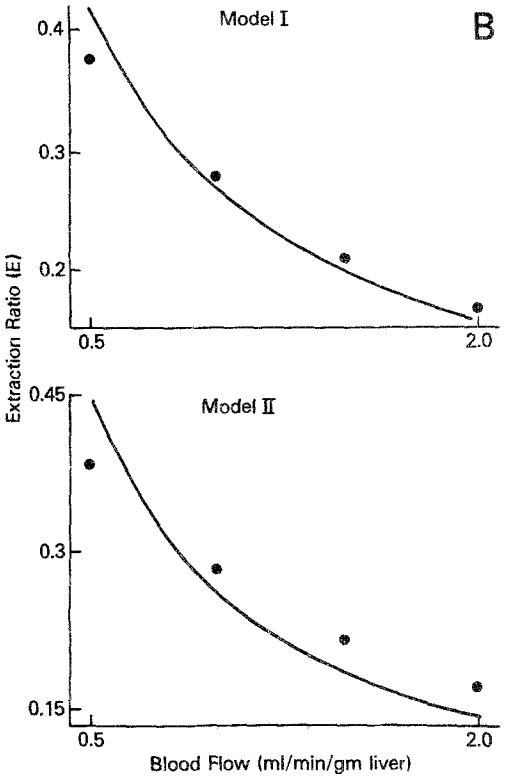

Fig. 12. Continued.

were fitted to equation 3 (hence model I) and equation 4 (hence model II) by NONLIN. A smaller sum of squares of residuals was obtained for model I. This statistic, however, is difficult to interpret, since both sides of equations 3 and 4 contain the same experimental variables, C_{In} and C_{Out}.

Physiologically, model II appears to be more appropriate than model I to describe hepatic drug clearance. One can easily conceive of a declining drug concentration along the length of the sinusoids with elimination of drug by hepatocytes lining the sinusoids. This plug flow situation is analogous to a steady-state plug flow reactor (25) where the material balance for a reaction component (metabolism) must be made for a differential element of volume or flow. This flow within the reactor is orderly, however, with no mixing occurring between any element ahead of and after it. There may be lateral mixing, but no mixing or diffusion along the paths. This pattern of circulation may be applied to most organs within the body, but not to the liver. The hepatic vasculature is a highly ramified network with cross-anastomoses, shunts, and indirect paths in addition to bulk flow. Any element of blood within one part of a sinusoidal bed can reach another part

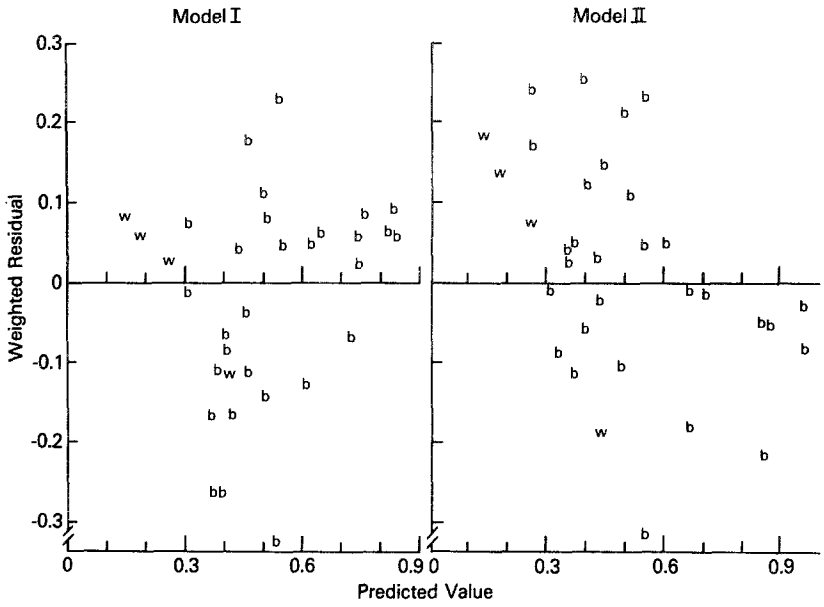

Fig. 13. Plot of the weighted residual against predicted value of the data from Brauer *et al.* (18) and Whitsett *et al.* (19) for models I and II. The symbols "b" and "w" represent the data from Brauer and Whitsett, respectively.

without leaving this section of the vascular tree. The portal vein, the hepatic artery, and the sinusoids are interconnected by both direct and obscure pathways (26). In this respect, the liver can be viewed operationally as a well-stirred compartment (model I), a situation analogous to a mixed reactor such that the exit stream (unbound drug concentration in emergent blood) has the same composition as the fluid within the reactor (unbound drug concentration in liver) (25). A similar assumption exists in the models used by Kety (27) and by Bischoff *et al.* (28), in that the effluent drug concentration is assumed to be in equilibrium with that within the tissues.

CONCLUSIONS

An attempt to discriminate between two models of hepatic drug clearance has elucidated that the "well-stirred" model is a better predictor of changes in clearance and in some other pharmacokinetic parameters with changes in hepatic blood flow. One cannot exclude the possibility, however, that other models of hepatic clearance exist that may fit the available data equally well, if not better. It must be borne in mind that,

while this "well-stirred" model is adequate to predict hepatic drug clearances, total body clearance and hence other pharmacokinetic parameters may deviate from our theoretical analysis (1) as one assumption used in defining the parameters is that the liver is the only eliminating organ. Changes in hepatic blood flow, binding within blood, and the hepatocellular activity of the enzyme system may or may not affect extrahepatic clearance to the same degree as hepatic clearance. Furthermore, gut wall metabolism has not been considered. These additional factors should be kept in mind when one applies the model to predict the influence of various factors on drug clearance and on other pharmacokinetic parameters.

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