Models of Hepatic Drug Elimination: A Response

Keyphrases □ Hepatic drug clearance—sinusoidal perfusion model, venous equilibrium model

To the Editor:

Although the distributed sinusoidal perfusion model of hepatic elimination described by Bass (1) appears more physiologically realistic than the two previous models, the statement that these latter models have been refuted experimentally should be challenged. First, the data on which this statement is based arise from only two studies (2, 3) and are not as conclusive as implied (1). The study design used in one of these studies (2) has already been acknowledged as not very useful for discriminating among the models in an earlier publication (4). In the other study (3) hepatic venous outflow concentrations of galactose were examined in the perfused rat liver under the influence of two perfusate flow rates (11 ml/min for 50 min, followed by 7 ml/min for 40 min, followed by 11 ml/min for 40 min). The sinusoidal models predict a lowering of the outflow substrate concentration at the lower flow rate, while the venous equilibrium model predicts no change in the outflow substrate concentration. A significant drop in outflow concentration was reported (3), but this was largely determined by averaging the data from the two periods of higher flow even though in about half the 10 experiments the outflow concentration during the second period was considerably greater than the outflow concentration during the first period.

One of the reviewers of this report has highlighted this fact by showing that a paired t test of outflow concentration during the two 11-ml/min flow periods, which were separated by 40 min of perfusion at 7 ml/min (or a constant flow of 11 ml/min in the three control studies), yields a statistically significant difference at the $p < 0.05$ level ($t = 2.19$ $DF = 12$). Similarly, when inflow concentration from these two time periods with a flow at 11 ml/min are tested, a statistically significant difference $p < 0.05$ ($t = 2.24$, $DF = 12$) is observed. One assumption necessary to ensure the legitimacy of all of the calculations performed by Bass is that the liver preparation is physiologically stable over the course of the experiment. Specifically, if a liver is infused with substrate at a constant rate, inflow and outflow concentrations should depend only on hepatic blood flow rate (*i.e.* V_{max} and K_m should not vary as a function of time).

Comparing the first two flow periods only (11 ml/min and 7 ml/min), during which the preparation is more reliable, the outflow of galactose concentrations actually increased in three experiments, decreased by no more than that of control livers (about 10%) in two livers, and decreased more substantially in the other five. Thus, the data could be viewed as inconclusive. Bass has also used the data from the earlier study (3) to support the distributed sinusoidal model (4) which predicts an increase in the logarithmic average of perfusate inflow and outflow concentrations of substrate with decreasing flow. The logarithmic average concentration, however, increased in only 5 of the 10 experiments, the mean change being 0.0009 mM ($SD = 0.0267$), a change of barely 1%: again not conclusive.

The other point that should be made is that the published data on lidocaine (5) supporting the venous equilibrium model have never been refuted by further experimentation with that drug. The studies with galactose cannot be assumed to automatically hold for all other substrates because the experiments were carried out under Michaelis–Menten conditions, galactose is an endogenous substance, and the zone of the liver in which a substance is eliminated (6) may dictate which model applies. For example, a substance, such as galactose, that is eliminated in the periportal region may be expected to follow the distributed sinusoidal model, whereas the venous equilibrium model may be appropriate for drugs that are eliminated in the centrilobular region where the enzymes for drug biotransformation are predominant (6). Therefore, in view of the functional hepatocellular heterogeneity it is naive to assume that all observed phenomena can be explained in terms of a single model as suggested (1). Models will, however, have served their function if they provide inspiration for further experimentation, as suggested previously (7), that ultimately results in more refined and physiologically meaningful models.

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Hepatic Extraction of Free Fatty Acids in Pregnant and Nonpregnant Female Rats

Keyphrases □ Free fatty acids—hepatic extraction □ Plasma protein binding—role of free fatty acids □ Hepatic drug clearance, intrinsic—role of drug protein binding and influence of free fatty acids

To the Editor:

Plasma protein binding can have important effects on the metabolic and excretory clearance of drugs (1, 2). Free fatty acids, whose concentrations in plasma can vary appreciably due to stress, diet, and other physiological variables (3), can competitively inhibit the plasma protein binding of many drugs (4, 5). Wiegand and Levy (6) have pointed out previously that extensive hepatic extraction of a protein binding inhibitor could cause an increase in the steady-state plasma concentration of unbound drug, with little or no effect on the concentration of total (free plus bound) drug, if the plasma protein binding of the drug

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Table I—Hepatic Extraction of Free Fatty Acids in Pregnant and Nonpregnant Female Rats [a]

	Pregnant			Nonpregnant		
	Venous Plasma Conc., μM		Hepatic	Venous Plasma Conc., μM		Hepatic
	Hepatic	Femoral	Extraction Ratio	Hepatic	Femoral	Extraction Ratio
Palmitic	87.1 ± 16.4	144 ± 28	0.391 ± 0.068	96.1 ± 12.4	149 ± 28	0.347 ± 0.087
Stearic	36.2 ± 7.8	51.4 ± 11.5	0.293 ± 0.078	44.9 ± 2.9[b]	54.1 ± 4.6	0.164 ± 0.102
Oleic	95.1 ± 14.6	170 ± 30	0.434 ± 0.081	105 ± 17	177 ± 43	0.391 ± 0.122
Linoleic	56.8 ± 10.7	113 ± 21	0.484 ± 0.113	76.2 ± 20.0	157 ± 51	0.504 ± 0.070

[a] Results are reported as mean ± SD, $n = 5$. [b] Significantly different from corresponding value in pregnant rats ($p < 0.05$).

is decreased with increasing inhibitor concentration. Such a change in drug concentration, apart from its pharmacodynamic implications (6), could be interpreted as a decrease in the drug's intrinsic clearance. Based on these considerations, we have determined the hepatic extraction of the four major endogenous free fatty acids in rats. Since the hepatic extraction of compounds is affected by their plasma protein binding and intrinsic hepatic clearance, studies were conducted also on pregnant rats, because pregnancy is associated with decreased plasma albumin concentration and decreased activity of certain oxidative metabolic processes (7, 8).

Female Sprague–Dawley rats (same age), both nonpregnant (≃200 g) and 20-days pregnant, had a cannula inserted in the femoral vein under ether anesthesia. The liver was then exposed through a midline abdominal incision. The liver lobules were reflected with wet gauze and the hepatic vein was clamped near its junction with the vena cava to avoid mixing blood from the liver with blood from the general circulation. Either 1 or 2 ml of blood was obtained from the hepatic vein by direct insertion of a 22-gauge hypodermic needle pointing toward the liver (9). Simultaneously, a blood sample from the femoral vein was obtained through the indwelling cannula. The blood was collected in plastic syringes containing EDTA (≃2 mg/ml of blood) and the plasma separated by centrifugation. The plasma was extracted into hexane within 5 min after blood collection, and the concentrations of the free fatty acids (as their methyl esters) were determined by GC by the method of Brunk and Swanson (10), but using smaller sample volumes and n-heptadecanoic acid rather than n-pentadecanoic acid as the internal standard.

The results of the study are summarized in Table I. The concentrations of the four major endogenous free fatty acids were considerably lower in hepatic venous plasma than in plasma from blood taken from the femoral vein. Linoleic acid was most extensively extracted, while the hepatic extraction of stearic acid was least pronounced. Plasma from pregnant rats is subject to rapid *in vitro* lipolysis which was minimized by rapid extraction of the plasma with organic solvent (11). Under these conditions the fatty acid concentrations in plasma of pregnant rats were similar to those in plasma of nonpregnant animals. There was no significant difference between pregnant and nonpregnant animals with respect to the hepatic extraction ratio of the individual free fatty acids. Hepatic vein–femoral vein albumin concentration ratios, determined in another experiment, were (mean ± SD) 1.03 ± 0.13, n = 4, for pregnant rats and 0.998 ± 0.056, n = 11, for nonpregnant animals.

The hepatic extraction values reported here are based on net concentration differences caused by hepatic extraction, and by hepatic output of free fatty acids synthesized by the liver (12). A contribution by extrahepatic tissues to the observed net concentration changes is also possible since solute concentrations in the femoral venous blood may not be the same as the concentrations in blood entering the liver (13). However, the femoral vein sampling site is appropriate because the usual pharmacokinetic studies involve blood sampling from a peripheral vein.

It has been reported (9) that ether anesthesia caused liver blood flow in rats to decrease from an average of 60.5 to 33.8 ml/min/kg. Unless ether anesthesia also causes a corresponding decrease in the intrinsic hepatic clearance of fatty acids, the extraction ratios reported here may be somewhat higher than the hepatic extraction ratios in nonanesthetized rats.

It is customary to determine the intrinsic clearance of drugs on the basis of the infusion rate and the steady-state concentration of unbound drug in plasma of blood taken from a peripheral vein. If the plasma protein binding of the drug is inhibited by free fatty acids in a concentration-dependent manner, then the free fraction of the drug in the blood entering the liver (and in the systemic circulation) is higher than that in blood leaving the liver, due to the decrease of the free fatty acid concentrations across the liver. Under these conditions, estimates of a drug's intrinsic clearance may be lower than the true value. This is particularly troublesome since free fatty acids are known to inhibit certain drug biotransformation processes (14, 15). An apparent decrease in the intrinsic clearance of a drug due to elevation of free fatty acids (5, 16) may represent the combined effects of metabolic inhibition and a change in plasma protein binding in the blood as it passes through the liver.

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Albumin Binding and Hepatic Uptake: The Importance of Model Selection

Keyphrases □ Albumin—effect on removal of taurocholate by the liver □ Taurocholate—removal by liver, albumin

To the Editor:

Dr. Colburn's letter to the *Journal* in March 1982 (1) purports to invalidate a conclusion we published earlier in the *Journal of Clinical Investigation* (2) concerning the role of albumin binding on the removal of taurocholate by the perfused rat liver. Having just learned of Colburn's communication we offer the following rebuttal. Better late than never!

The observations we reported (which are not in contention) show that the extraction fraction of taurocholate declines only slightly when the perfusate albumin concentration is increased, even though this maneuver reduces the free (unbound) fraction of taurocholate by a factor of five. The table shows the data for rat livers perfused at the same flow rate and with the same total concentration of taurocholate (18 μM).

Albumin Concentration (g/dl)	Free Fraction of Taurocholate in Perfusate	Taurocholate Extraction Fraction
0.5	0.57	0.97
5.0	0.11	0.86

The objective of the experiment was to learn what these numbers imply about the apparent rate constant for hepatic uptake, given that the low concentration of taurocholate ensures that both the binding reaction in extracellular fluid and the removal process are operating far removed from saturation and that the binding reaction is fast enough compared with the removal rate to be considered at equilibrium. These stipulations are also not in contention. Instead the controversy focuses on the choice of an appropriate model with which to interpret the data.

Colburn prefers to model the extracellular fluid as a single homogeneous compartment in which each liver cell is exposed to the same taurocholate concentration—the so-called "lumped" or "venous equilibrium model." In this case the steady-state conservation equation is:

$$Fu_0 = \psi VKu_v + Fu_v \qquad \text{(Eq. 1)}$$

in which ψ is the free fraction, F is perfusate flow, K is the rate constant for removal of free taurocholate, and V is the extracellular volume. The terms, u_0 and u_v, in Eq. 1 are the inflow and outflow concentrations of total taurocholate, respectively. Rearranging Eq. 1 yields:

$$K = FE/[\psi V(1 - E)] \qquad \text{(Eq. 2)}$$

in which the extraction fraction, E, is $(u_0 - u_v)/u_0$. Equation 2 is the one suggested by Wilkinson and Shand (3) to whom Colburn appeals for support.

We have preferred to use a so-called "distributed" model accounting for the decline in taurocholate concentration that occurs along each sinusoid. The conservation relation for a single sinusoid is in this case:

$$F\frac{du}{dx} = -\psi\gamma Ku \qquad \text{(Eq. 3)}$$

where x is the sinusoidal volume running from $x = 0$ at the portal inlet to $x = V$ at the hepatic venous outlet and γ is the ratio of the sinusoidal volume to the volume of the Disse space divided by the sinusoidal volume. The solution to Eq. 3 is:

$$K = -F\ln(1 - E)/\psi\gamma V \qquad \text{(Eq. 4)}$$

If one now computes the ratio of the K values from the observations made with high and low concentrations of albumin, the results are strikingly different depending on the choice of the model.

$\frac{K \text{ at high albumin}}{K \text{ at low albumin}}$	Lumped Model	Distributed Model
	0.98	2.9

The interest in these calculations derives from the fact that both models are constructed on the conventional teaching that only free taurocholate is available for removal. If we accept Colburn's model this assumption appears confirmed because the calculations yield the expected identity of the rate constants. If we accept the distributed model, however, the data contradict the conventional teaching because in this case the rate constants differ by nearly a factor of three. The direction of the discrepancy is such that liver cells appear to enjoy some special mechanism for enhancing the dissociation of the albumin–ligand complex—in effect making more free taurocholate available to liver cells than the conventional teaching would predict. There is, in fact, a growing body of additional evidence to support this conclusion (4–7), but our concern here is with the question of which model to accept.

Those who choose the Colburn model will have to decide where the change from u_0 to u_v occurs. Plainly it cannot be attributed to the removal of taurocholate by hepatocytes because the model requires that all liver cells be exposed to the same concentration. Alternative choices that the drop in concentration occurs in the presinusoidal portal circulation or in the postsinusoidal hepatic veins would not only be anatomic nonsense but would imply that the calculated rate constant has nothing whatever to do with the transport function of liver cells. On this basis we conclude that although Colburn's analysis of the data appears to confirm a widely held preconception, it is physiologically irrelevant. His model simply does not describe a real liver.

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