and the subsequent increase in free I plasma concentrations, which saturate the uptake process in a manner similar to large doses of I.

Biliary excretion data obtained from rats (8–11) have often been of sufficient quality to enable the pharmacokinetic support of intrahepatic models such as that suggested above. Although our data was quite limited, it seems to suggest that data of sufficient quality may be difficult to obtain from dogs. The fact that the biliary excretion half-life was found not to vary with dose supports our contention that the changing plasma half-life reflects a saturable hepatic uptake rather than an elimination process.

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Effect of Nonuniform Bile Flow Rate on the Rate of Biliary Excretion of Bromophenol Blue in the Beagle

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Abstract □ Following the intravenous administration of bromophenol blue to beagle dogs, graphs of the biliary excretion rate *versus* time displayed drastic fluctuations which render them of little value for standard pharmacokinetic modeling purposes. It was shown that these fluctuations in excretion rate are highly correlated with corresponding fluctuations in the bile flow rate. An expression was derived which accounts for the primary effect of nonuniform bile flow rate on the biliary excretion rate. This treatment would enable the use of such biliary excretion rate data for pharmacokinetic modeling. Secondary effects of nonuniform bile flow on the biliary excretion rate are also discussed. It is suggested that the modeling of other flow rate-dependent elimination processes could benefit from such a treatment.

Keyphrases □ Bromophenol blue—biliary excretion, nonuniform bile flow rate, dogs □ Biliary excretion—bromophenol blue, dogs, nonuniform bile flow rate □ Bile flow rate—nonuniform, biliary excretion of bromophenol blue, dogs

Biliary excretion has long been a subject of intensive investigation (1). Within the past decade, there has been an increase in the number of studies in which the bile duct has been cannulated to permit the determination of drugs and metabolites in the bile. These studies typically involve collecting bile samples at relatively long intervals and, usually, the cumulative amounts of drug excreted are reported as a function of time (2–5). These studies have been particularly useful in assessing the magnitude of the first-pass effect and the role of hepatobiliary elimination in the overall elimination of a wide variety of compounds (1). More recently, it has been shown that high-quality bile data can provide pharmacokinetic support for an intrahepatic model for hepatobiliary elimination in rats (6, 7). Takada *et al.* (7) obtained biliary excretion rate data (mg/h *versus* time) of sufficient quality to enable a statistically significant fit to a tetraexponential function, which they identified with a five-compartment model for hepatobiliary transport.

The possibility of obtaining similar data in higher animals is appealing, yet the problem must be viewed somewhat pessimistically due to the fact that the bile flow rate fluctuates

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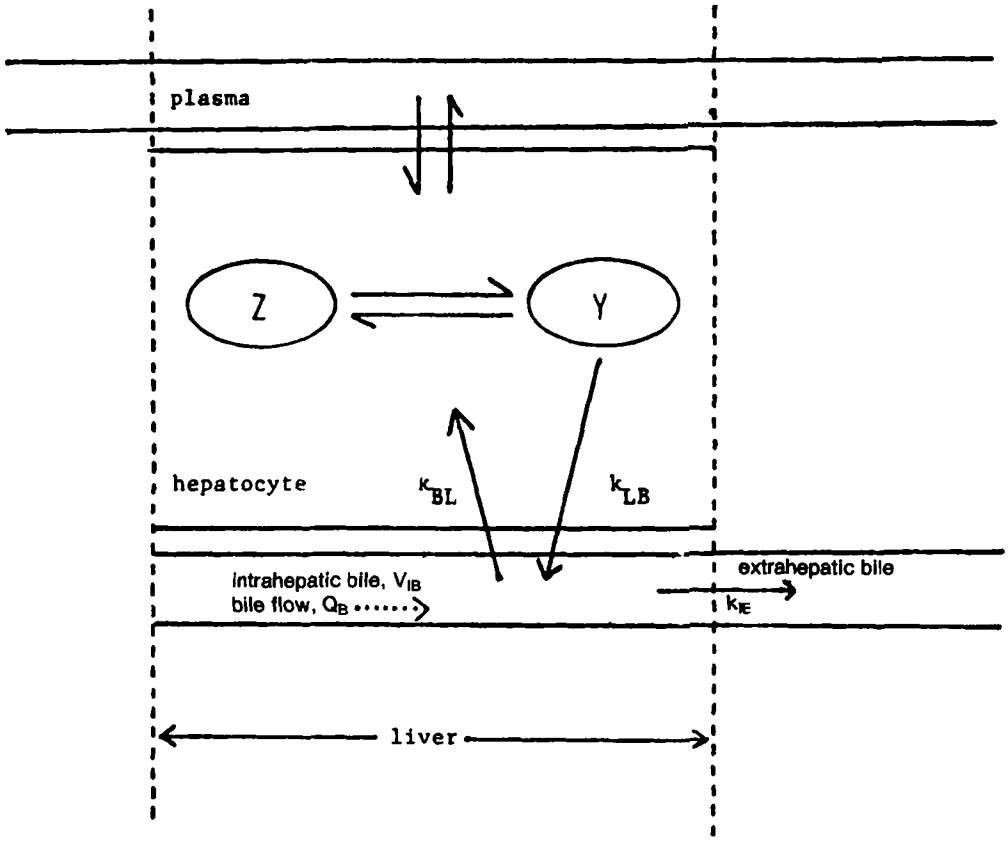

Figure 1—*Schematic representation of physiological transport and elimination via the hepatobiliary route. Y and Z represent two liver protein fractions which are involved in hepatobiliary transport (8).*

more markedly in higher animals than in the rat. The purpose of this brief communication is to present preliminary results which confirm that nonuniform bile flow rates seriously complicate the interpretation of biliary excretion rate data in the beagle dog. Possible explanations for the observed correlation between excretion rate and bile flow rate, as well as the basis for the development of a bile flow rate-dependent model for hepatobiliary transport, will be discussed.

THEORETICAL SECTION

To best treat excretion rate data, it will be necessary to develop a physical model of hepatobiliary transport which explicitly involves the biliary flow rate. Figure 1 depicts a simple model, similar to that suggested by Takada *et al.* (7) in that it distinguishes between intrahepatic and extrahepatic bile. The shaded region is intended to represent the bile canaliculus in contact with the hepatocytes. Let V_{IB} symbolize the volume of intrahepatic bile contained within the canaliculi and A_{IB} represent the amount of drug in intrahepatic bile. The transport of drug between the hepatocyte and intrahepatic bile is represented by k_{LB} and k_{BL}. If the bile production rate or flow rate, Q_B, were zero, then no drug would be excreted from the liver. The rate at which drug is actually excreted depends on the rate at which drug is eluted from the intrahepatic bile compartment, of volume V_{IB}, by newly produced bile flowing at a rate Q_B:

$$\frac{dA_{EB}}{dt} = k_{IE}A_{IB} = \frac{Q_B}{V_{IB}} \cdot A_{IB} \qquad \text{(Eq. 1)}$$

where A_{EB} is the cumulative amount of drug excreted in bile as a function of time and k_{IE} is the rate constant for transport from intrahepatic bile to extrahepatic bile[1]. In the event that Q_B is observed to vary between sample times, Eq. 1 could, as a first approximation, be assumed to hold over the individual sampling intervals. Thus, by dividing the fluctuating excretion rate by the fluctuating bile flow rate, estimation of pharmacokinetic parameters can be achieved by fitting the quotient to an expression for A_{IB}:

$$\frac{\text{excretion rate}}{\text{flow rate}} = \frac{(\Delta A_{EB})_j/\Delta t_j}{(Q_B)_j} = \frac{1}{V_{IB}} \cdot (A_{IB})_j \qquad \text{(Eq. 2)}$$

In Eq. 2, j is an index referring to the sampling interval, Δt, and V_{IB} is treated as a constant model parameter to be determined.

RESULTS

As described previously (9), bromophenol blue (I) was administered intravenously to three beagle dogs after they had each recovered from cannulation surgery. During surgery, the common bile duct was cannulated to permit collection of the total bile excreted and the gallbladder was removed to avoid bile storage and thus permit collection as the bile was produced. Bile salt dietary supplements[2] were given to maintain the body weight of each dog throughout the study.

The limited data obtained to date is shown in Figure 2a–d. The solid lines connect the biliary excretion rates of I plotted at the midpoints of the collection intervals and the dashed lines connect the corresponding bile flow rates. It is apparent that the fluctuations in the excretion rate correspond to fluctuations in the bile flow rate. It is also apparent that this excretion rate data is unsuitable for pharmacokinetic modeling in the present form.

By mathematically pooling successive samples, the rate plots can be smoothed to a considerable extent as shown in Fig. 3. In Fig. 3, the pooled collection intervals were 30 and, later, 60 min compared with actual collection intervals of 5, 10, and 15 min. Pooling the data smoothed the curve by replacing the rapidly fluctuating bile flow rate with an average flow rate. We should be somewhat critical of attempts to fit smoothed curves which are obtained by masking rather than explaining the details of the data. It should be noted that these details are analogous to excretion data when collected at longer sampling intervals.

Using Eq. 2, values for excretion rate divided by flow rate were generated and plotted, along with the excretion rate, against time (Fig. 4). It is apparent that this new treatment of bile data smoothed the curves to the extent that pharmacokinetic evaluation may be possible.

[1] Although the relationship $k_{IE} = Q_B/V_{IB}$ is discussed in any classical treatment of chromatography, the interested reader is referred to a pharmacokinetic application discussed by Gumtow *et al.* (8).

[2] Reheis Chemical Co., Tarrytown, N.Y.

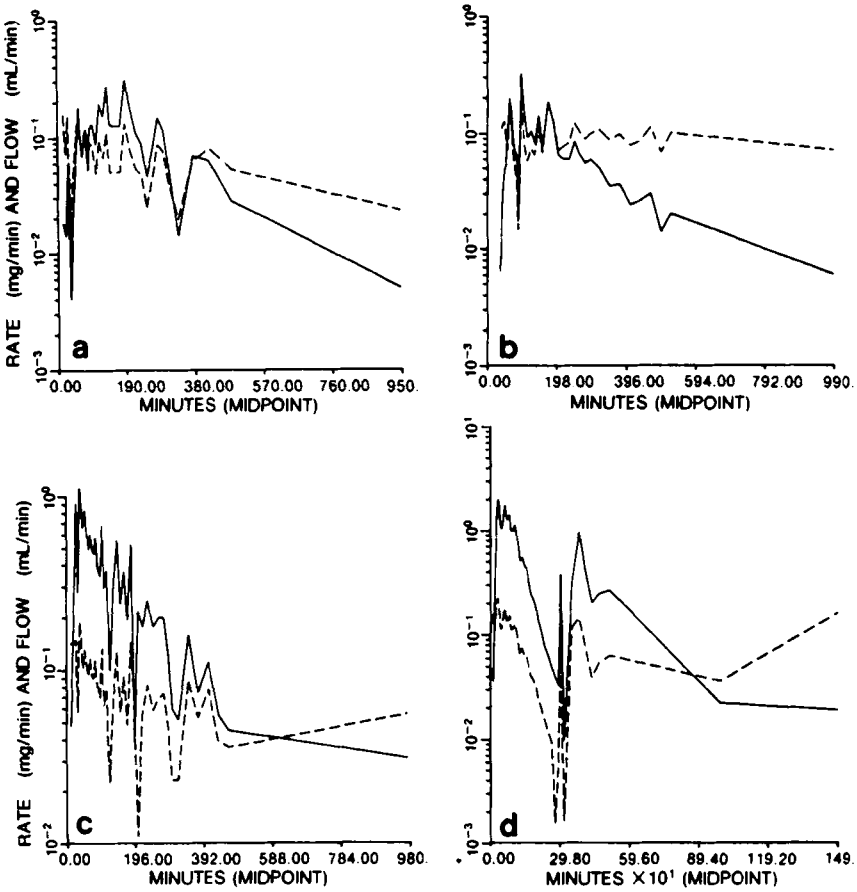

Figure 2—*Biliary excretion rate (—) and bile flow rate (- - -) of I following intravenous bolus injections of 5 mg/kg to dogs B (a) and C (b) and 20 (c) and 30 mg/kg (d) to dog C.*

DISCUSSION

Before addressing this apparent bile flow rate dependence, we note that limited information can be extracted from this data in a variety of ways. The excretion of drug in bile is somewhat analogous to the excretion of drug in urine, breast milk, sweat, saliva, and lacrimal fluid. Experimentally, we measure the accumulation (and rate of accumulation) of the amount of excreted drug in the excreted fluid, rather than the concentration, because the volume of the excreted fluid is changing. This could be contrasted with the treatment of plasma data, in which case we monitor the decreasing concentration of drug in an essentially constant volume of fluid.

In the preceding paper (9), we presented plots of the cumulative amount of bromophenol blue excreted as a function of time to indicate the extent to which various doses are eliminated through the bile, and we noted that σ^- plots of the same data seemed to suggest that the observed dose-dependent plasma elimination is due to a dose-dependent liver uptake rather than to a hepatobiliary elimination process. Both types of plots mask not only the random variability but the genuine detail (*i.e.*, fluctuations) in such excretion data (10). Excretion rate plots that provide a more precise presentation of the data are normally preferred, but as shown here, these may not be adequate for pharmacokinetic modeling.

In contrast, the data generated by Eq. 2 accounted for most of the effect of nonuniform bile flow rate and yield a graph to which one could fit a model-based expression for A_{1B}. It might be noted that the A_{1B} curve indicated in Fig. 4 is of the same shape as a corresponding curve (for X_4) that Takada *et al.* (7) simulated for a compartmental model which could be used to describe the physiological transfer processes indicated in Fig. 1. It should also be noted

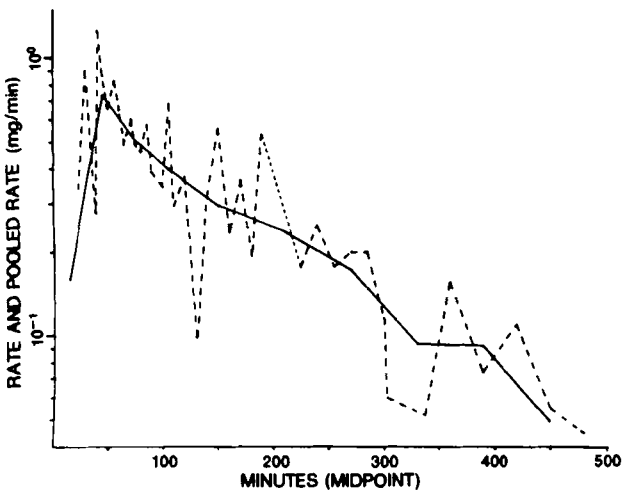

Figure 3—*Actual (- - -) and mathematically pooled (—) biliary excretion rate data for I following an intravenous bolus injection of 20 mg/kg. The small segment (· · ·) indicates missing data.*

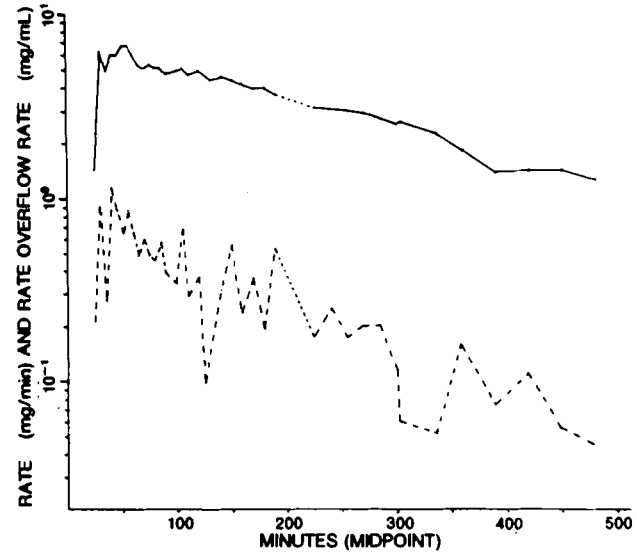

Figure 4—*Biliary excretion rate (- - -) and biliary excretion rate divided by bile flow rate (—) of I following intravenous bolus injection of 20 mg/kg. The small segments (· · ·) indicate missing data.*

that the fitting is complicated by the fact that A_{1B} is also dependent on k_{1E}, although far less markedly than A_{EB}.

We have chosen not to attempt such pharmacokinetic modeling at the present time, not only because of the incompleteness of our data but because additional analyses of the effects of nonuniform bile flow rate should be undertaken. It certainly appears that the primary effect of nonuniform bile flow rates is on k_{1E} and that our treatment of this effect describes the data. However, there are secondary effects which should also be addressed.

One example would be the effect of bile flow rate on the thickness of the diffusion layer adjacent to the bile canalicular membrane, which represents an additional barrier to the transport between the hepatocyte and intrahepatic bile. The diffusion layer thickness within a cylindrical tube is discussed by Levich (11) and it can be shown that $\delta \alpha v_0^{-1/3}$ (9), where δ is the diffusion layer thickness and v_0 is the maximum flow velocity at the axis of the tube. For a nonelastic tube of constant radius, v_0 is directly proportional to the flow rate, Q_B. Thus, passive diffusion between the hepatocyte and the canaliculi would proceed at a rate proportional to $Q_B^{1/3}$.

Nonuniform bile flow rates result in nonuniform hydrostatic pressure within the canaliculi (12), which, in turn, could affect the effective diameter of any pores existing in the canalicular membrane. If the transport between hepatocyte and intrahepatic bile involves pore filtration (12), then this secondary effect of nonuniform bile flow rates could become important. The relationship between pore size and pore filtration rate is discussed by Lakshminarayanaiah (13).

Lightfoot (14) discusses flow through elastic ducts and indirectly indicates the effect that flow rate might have on the rate of transport across the duct wall. It could also be pointed out that the elution approach discussed above is quantitatively correct only if there is perfect mixing within the intrahepatic bile compartment.

All of the secondary points mentioned above suggest that further analysis is required before biliary excretion data can be used to accurately describe a model for hepatobiliary uptake and elimination. However, if the objective is merely to use biliary excretion data to support a crude model which could be used for predictive (*i.e.*, dosing) purposes, then our treatment of nonuniform bile flow rate should be of some value.

Lastly, it should be noted that the elution approach discussed above could possibly be applied to other flow rate-dependent physiological processes. Whereas the effects of blood flow rate have received considerable attention in the pharmacokinetic literature, the effects of nonuniform flow rates on elimination *via* urine, milk, saliva, lacrimal fluid, *etc.* should account for some of the fluctuation or scatter frequently observed in that type of data.

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Radiotelemetric Method for Evaluating Enteric Coatings *In Vivo*

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Received April 4, 1983, from *INTERx, Merck Sharp & Dohme Research Laboratories, Lawrence, KS 66044.* Accepted for publication July 20, 1983. *Present address: College of Pharmacy, University of Michigan, Ann Arbor, MI 48109.

Abstract □ A radiotelemetric method for the *in vivo* evaluation of enteric coating performance is described, and its advantages and disadvantages are compared with those of other available methods. Hydroxypropyl methylcellulose phthalate was used as the test enteric coating. Four dogs were administered several batches of enteric-coated tablets containing buffers. Tablet disintegration was determined by radiotelemetric detection of the pH drop in the upper intestine due to release of the buffer. Premature rupture of the coating in the stomach was detected by a rise and then a fall in gastric pH prior to gastric emptying. The average gastric emptying time was 80 ± 18 min (*SEM*), while the average time for a tablet to disintegrate in the upper intestine was 14.2 ± 2 min. The average disintegration time was not affected by a change in the batch (for a given tablet core pH) or the dog used, suggesting that the method yielded readily reproducible results. Although there was little correlation with *in vitro* disintegration times, the method gave results similar to those reported in the literature for the same enteric coating in a human study. Of the formulations tested, it was concluded that buffering the core to pH 4 was most suitable for studying enteric coating performance.

Keyphrases □ Enteric coating—*in vivo* disintegration, radiotelemetry, hydroxypropyl methylcellulose phthalate □ Hydroxypropyl methylcellulose phthalate—*in vivo* disintegration, tablet coating, radiotelemetry □ Radiotelemetry—*in vivo* disintegration, hydroxypropyl methylcellulose phthalate tablet coating

Enteric coating of dosage forms has been used in several ways to improve drug delivery. For example, the bioavailability of acid-labile drugs such as erythromycin can be improved by avoiding exposure of the drug to the gastric contents (1). A second reason for using an enteric coating is to avoid gastric irritation caused by drugs such as aspirin (2). Enteric coating has also been used to delay the release of a drug taken at bedtime with the aim of ensuring therapeutic blood levels when the patient awakes (3).

Several methods are available for evaluating enteric coatings. A widely used *in vitro* test is the USP disintegration test for enteric-coated tablets (4). *In vivo* methods of following the

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