0022-3565/89/2493-0869$02.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

Vol. 249, No. 3
Printed in U.S.A.

Zonal Compartmentation of Perfused Rat Liver: Plasma Reappearance of Rhodamine B Explained

INEKE BRAAKMAN,[1] GENY M. M. GROOTHUIS and DIRK K. F. MEIJER
Department of Pharmacology and Therapeutics, University of Groningen, Groningen, The Netherlands
Accepted for publication February 27, 1989

ABSTRACT

Rhodamine B (RB) fluorescence reappears in perfusion medium of a cyclically perfused rat liver after a rapid initial removal phase. At the same time the compound redistributes in the liver from acinar zone 1 toward zone 3. By analysis of the metabolic profile of RB, and by inhibition of glucuronidation (the main metabolic route) with salicylamide, we show in this paper that formation and secretion of RB-conjugates from liver into perfusate is not involved in the reappearance and redistribution phenomena. We therefore sought the explanation in a kinetic model, in which the acinar heterogeneity of the liver was simulated by several sequential liver compartments. Most kinetic parameters we used in the simulation were calculated from previous experiments with RB (Braakman *et al.*, Hepatology **7:** 849–855, 1987). This led to an accurate simulation of the measured RB curves in bile, medium and the acinar zones of the liver. In this study we show that a secondary rise in medium concentration of an injected compound is not necessarily caused by metabolism, but can be easily explained by considering the liver a sequence of compartments, instead of one well-stirred compartment. The conditions for the reappearance as well as for the intrahepatic redistribution are: a fast uptake into the liver, combined with a fast sinusoidal secretion and a slow biliary excretion of the injected substance.

When a drug is injected i.v. into an organism, the plasma concentration of the drug will fall with time if this compound is distributed to any kind of tissue. Usually liver and kidney accumulate substantial portions of the dose, as a first step to eliminate the compound from the body. To investigate the events during hepatic processing of a drug, the perfused rat liver preparation is a highly versatile technique (Gores *et al.*, 1986; Wolkoff *et al.*, 1987). Many compounds taken up by the perfused liver are metabolized to more hydrophilic derivatives, which can be secreted from the liver, either into bile or back into the perfusate. Often such metabolites markedly differ physicochemically from the parent compound, but the metabolic conversion remains undetected because of the unspecific detection methods used (for instance fluorometry when the compound and metabolite both are fluorescent or scintillation counting if the drug is labeled with a radioactive isotope). If formation of the metabolite is rapid compared to secretion, a rise in total plasma concentration of fluorescence or radioactivity can occur, after the initial decline. Such a rise in plasma concentration of total drug is usually interpreted to be the result of some kind of metabolism of the administered drug.

In a previous study (Braakman *et al.*, 1987) we reported the reappearance of RB in the perfusion medium although no evidence of major metabolism of the drug could be obtained. Indirect morphological evidence pointed to a hitherto unrecognized transport from hepatocytes in zone 1 to hepatocytes in zone 3. In this paper we present a kinetic model explaining this phenomenon. In this model the liver consists of two or more sequential compartments instead of one, representing an acinar zonation of the liver (Rappaport *et al.*, 1954). By means of computer simulation studies we now show that a rising plasma concentration can be explained by time-dependent intrahepatic redistribution, and does not necessarily involve intracellular metabolism of the injected drug.

Materials and Methods

Materials. Male Wistar rats (220–290 g) were fasted overnight before use. RB and SAM were obtained from Sigma Chemical Co. (St. Louis, MO). All other chemicals were purchased from sources described before (Braakman *et al.*, 1987).

Isolated perfused rat liver. Each liver was prepared surgically, connected to the perfusion apparatus and maintained at 38°C as described previously (van der Sluijs *et al.*, 1986; Meijer *et al.*, 1981). Livers were perfused with 100 ml of recirculating Krebs-Henseleit-bicarbonate buffer, containing 10 mM glucose and 150 μM bovine serum albumin, at a hydrostatic pressure of 10 cm. Perfusion rate was adjusted to 3.5 ml/min/g of liver, to ensure adequate O_2 supply; pH was maintained between 7.35 and 7.45, either by slight adjustments in pCO_2 or by the addition of 0.15 M $NaHCO_3$.

Received for publication March 22, 1988.
[1] Present address: Department of Cell Biology, Yale University School of Medicine, 333 Cedar Street, New Haven CT, 06510.

ABBREVIATIONS: RB, rhodamine B; SAM, salicylamide; TLC, thin-layer chromatography; ECV, extracellular volume; R_f, relative running distance; FITC, fluorescein isothiocyanate.

Experimental design. Livers were perfused for 120 min. RB was injected as a single bolus of 120 nmol after a stabilization period of 30 min. Bile and perfusate samples were collected at various time intervals. Bile volume was determined gravimetrically, assuming a density of 1 g/ml. To study the influence of glucuronidation on RB-kinetics, SAM was injected in a single dose of 500 μmol at the same time as RB was injected.

Analytical methods. RB and metabolites were determined and identified in bile, perfusate and liver homogenates as described previously in detail (Braakman *et al.*, 1987). Recovery of extraction of RB was checked by adding RB to blank samples and treating them as experimental samples. Recovery was always 100%. Glucuronic acid conjugates were identified by incubation with β-glucuronidase and subsequent fluorescence analysis. Samples from perfusate, bile, liver homogenate and β-glucuronidase incubation mixtures were prepared for TLC as described before (Braakman *et al.*, 1987). RB added to blank samples before spotting them on a TLC plate displayed the same behavior as in experimental samples. TLC plates were developed for 2.5 hr in a chamber saturated with 80% *n*-propanol-20% formic acid. SAM was determined according to Levy and Matsuzawa (1967).

Fluorescence microscopy studies. Frozen liver sections of 8 μm were cut in a cryostat (−25°C) and examined as described before (Braakman *et al.*, 1987). Livers were perfused with RB for 10, 20, 30, 40, 50 or 60 min, with or without SAM.

Statistical methods. Comparison between two means was made with Student's *t* test, after checking equality of variances with an *F* test (Snedecor and Cochran, 1980). Values are expressed as means ± S.E. with $P \leq .025$ considered significant.

Computer simulations. Simulation studies were done on an Atari 1024 ST personal computer. A compartmental model was designed, in which the liver consists of several compartments in sequence, instead of one well-stirred compartment (fig. 2). In this model, the central medium compartment is represented by compartment M_{11}, with a volume of 100 ml as in our perfusion set-up. M_1 to M_{10} are small medium compartments in the liver, together representing the ECV: sinusoids and Disse space, with a total volume of about 1.5 ml (Sasse, 1986). L_1 to L_{10} are the sequential liver tissue compartments, S_1 to S_{10} are the corresponding intracellular sequestration sites and B represents bile. To simplify the model, biliary excretion was defined as the sum of metabolism and excretion. The amount of RB transported per time period from one compartment to another was calculated for each possible transport route (each arrow in fig. 2); *e.g.*, the amount transported from compartment *X* to compartment *Y* ($\Delta A_{x\rightarrow y}$) was calculated as

$$\Delta A_{x\rightarrow y} = k(XY)\cdot A_x \cdot \Delta t$$

in which $k(XY)$ is the rate constant of transport from *X* to Y; A_x is the amount of RB in *X* and *t* is the time interval. The amount of RB in compartment *X* at time *t* ($A_{x,t}$) was found by adding to $A_{x,t-1}$ every amount transported *to* compartment *X* ($A_{\rightarrow x}$) and by subtracting every amount transported *from* compartment *X* ($A_{x\rightarrow}$). So, if all parameters "*k*" are defined, a complete amount of RB *vs.* time curve can be calculated for every compartment in the model. At time zero we started with the amounts in all compartments near zero (nanomoles), except for the central medium compartment (M_{11}), which contained 120 nmol (=100%). The curves were calculated for 90 minutes postinjection.

Results

Kinetic and metabolic pattern of RB. The plasma disappearance curve of RB in an isolated rat liver perfusion is shown in figure 1A. The initial clearance of RB was close to the perfusion flow; consequently, hepatic extraction approached 100% (Braakman *et al.*, 1987). Ten minutes after injection the medium concentration started to rise again, until a plateau was reached. Biliary excretion of RB was low, only about 9% of the injected dose was excreted in 90 min (fig. 1B).

With TLC we investigated the metabolic profile of RB in medium, liver and bile. The results are shown in table 1. In medium and liver homogenates, only authentic RB could be recovered ($R_f = 0.85$); no metabolites were detected, the detection limit being 20 pmol of RB. In bile, however, several metabolites appeared, of which the acyl-type-RB-glucuronide ($R_f = 0.61$) quantitatively was the most important one. This glucuronide was identified by incubation of the samples with β-glucuronidase, whereupon the spot at $R_f = 0.61$ disappeared and the RB-spot ($R_f = 0.85$) grew more intense. The incubation mixture was assayed for RB fluorescence before and after incubation. Fluorescence intensities and spectra were identical. The TLC plate revealed that before incubation the glucuronide was the main component whereas afterward RB was present. So, apparently quantum yield and fluorescence spectrum of RB and its glucuronide are similar.

Influence of SAM. Although no metabolite was present in medium and liver, the possibility remained that the acylglucuronide still was formed and then hydrolyzed in the liver by esterases or a slightly alkaline pH. In that case the intra-acinar redistribution and the kinetics of RB could be determined markedly by metabolism. To exclude definitively that metabolism was the cause for the rise in medium fluorescence, we inhibited glucuronidation with SAM (Howell *et al.*, 1986; Boutin *et al.*, 1984). The disappearance of SAM from the perfusion medium was monophasic with a half-life of 140 min, so the compound was present at a relatively constant concentration during the whole experiment. TLC-data reveal that the gluc-

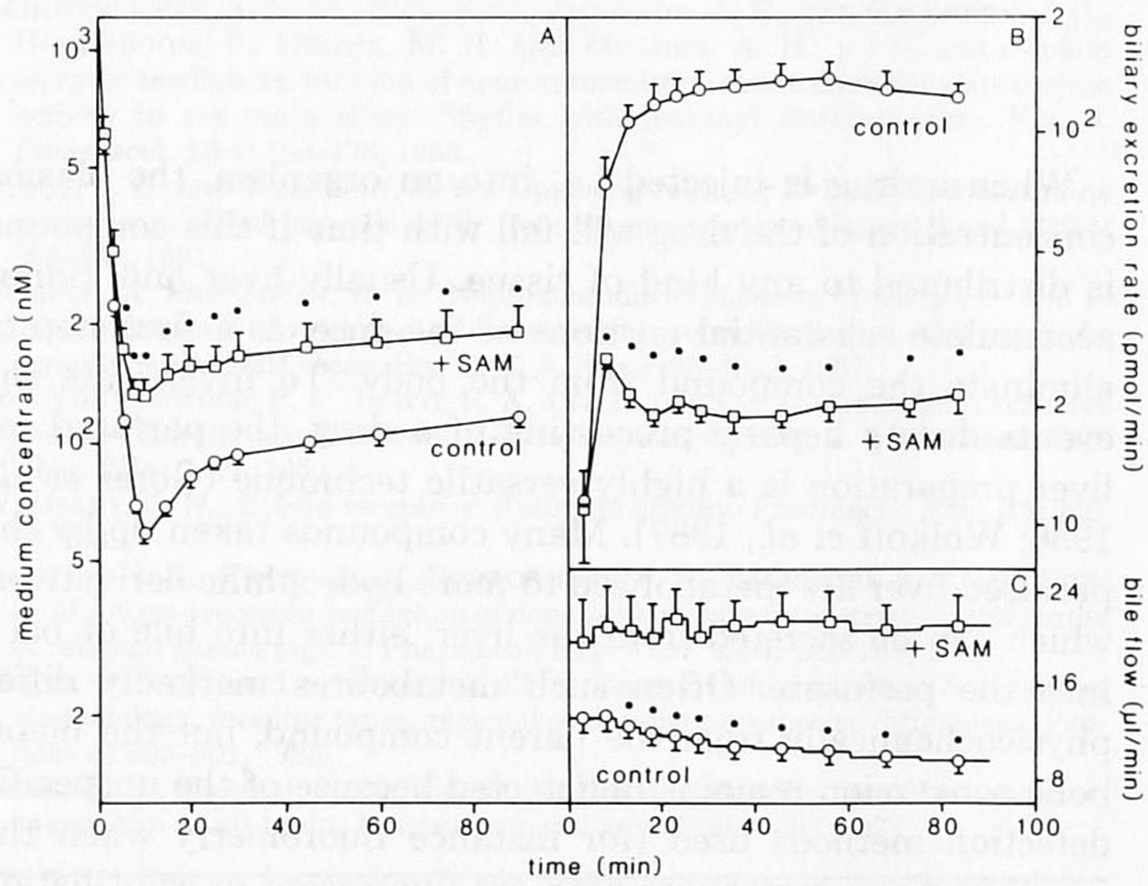

Fig. 1. Medium concentration (A), biliary excretion rate (B) and bile flow (C) *vs.* time curves of 120 nmol of RB injected into the medium of a recirculating isolated liver perfusion. Controls (○, $n = 4$); with 500 μmol of SAM (□, $n = 3$). Data are means ± S.E. *Student's *t* test; $P \leq .025$.

TABLE 1

Influence of SAM on the metabolic profile of RB

R_f	Color[a]	Compound	Control			With SAM		
			Medium	Bile	Liver	Medium	Bile	Liver
0.85	Orange	RB	+[b]	+	+	+	+	+
0.69	Yellow	Phase I-metabolite[c]	−	+	−	−	+	−
0.66	Orange/yellow	Phase I-metabolite	−	+	−	−	+	−
0.61	Orange	RB-glucuronide	−	+	−	−	−	−
0.53	Yellow	Phase I-metabolite	−	+	−	−	+	−

[a] Observed at 365 nm.
[b] −, not detectable (below 20 pmol); +, detectable.
[c] Webb and Hansen, 1961.

uronide indeed was not formed in the presence of SAM (table 1); it was detected neither in bile, nor in medium, nor in the liver. Biliary excretion diminished from 9 to 1.4% of the dose in 90 min (*cf.* fig. 1B), although bile flow was twice as high as in controls (fig. 1C). The excretion of RB and the other metabolites remained the same as in controls.

Figure 1A shows that, in the presence of the inhibitor, the secondary rise in medium concentration still occurred. The RB concentration was statistically significantly higher after 6 min, compared to the control situation (8.5% of the injected dose of RB in controls *vs.* 14.5% with SAM at 90 min after injection).

Intrahepatic distribution of RB was assessed by microspectrofluorometric microscopy. In controls (Braakman *et al.*, 1987), 10 min after injection the fluorescence was localized mainly in the first half of the acinus. Fifty minutes later, fluorescence was shifted toward zone 3, with a higher intensity in zone 3 than in zone 1. The inhibitor did not change this time-dependent redistribution of RB from zone 1 to zone 3, except for a slight acceleration of the shift.

Computer simulations. To explain our observations we propose a kinetic compartmental model, in which the liver consists of several compartments in sequence (fig. 2). Rate constants were evaluated as first order because a 10-fold increase in RB dose did not change our observations (results not shown). Simulations were carried out after adding a value to each rate constant $k(XY)$ in the model. The choice of these values was limited by some considerations. M_{11} is much larger than $\sum_{i=1}^{10} M_i$ (=ECV); therefore $k(M_{11}M_1)$ had to be much smaller than the other "medium flow" parameters $k(M_iM_{i+1})$. At the same time uptake was very fast compared to medium flow, so $k(M_iL_i)$ had to be much larger than $k(M_iM_{i+1})$. On the other hand, redistribution towards zone 3 was fast too (Braakman *et al.*, 1987), so $k(L_iM_i)$, although smaller than $k(M_iL_i)$, had to be large as well.

We simulated acinar heterogeneity into this model, by regarding $M_1+M_2+M_3$ as the ECV of zone 1, $M_8+M_9+M_{10}$ as the ECV of zone 3 and the first and the last three liver and sequestration compartments as liver tissue zone 1 and zone 3, respectively. For the rate constants for uptake, secretion and sequestration of the zones, in these simulations we used the ratio between zone 1 and zone 3, as calculated in earlier experiments with RB (Braakman *et al.*, 1987). The zone 1/zone 3 ratios we used were: for $k(M_iL_i)$ 1, for $k(L_iM_i)$ 0.73, for $k(L_iS_i)$ 0.62, for $k(S_iL_i)$ 1.78 and for $k(L_iB)$ 1.39.

A simulation resulted in the curves depicted in figure 3. The parameter values used are in the legend. Figure 3A shows that for this set of parameters the simulated medium curve was identical to the experimentally measured curve. The curves for the first three and the last three liver compartments (reflecting "zone 1" and "zone 3," respectively; fig. 3B) agreed nicely with

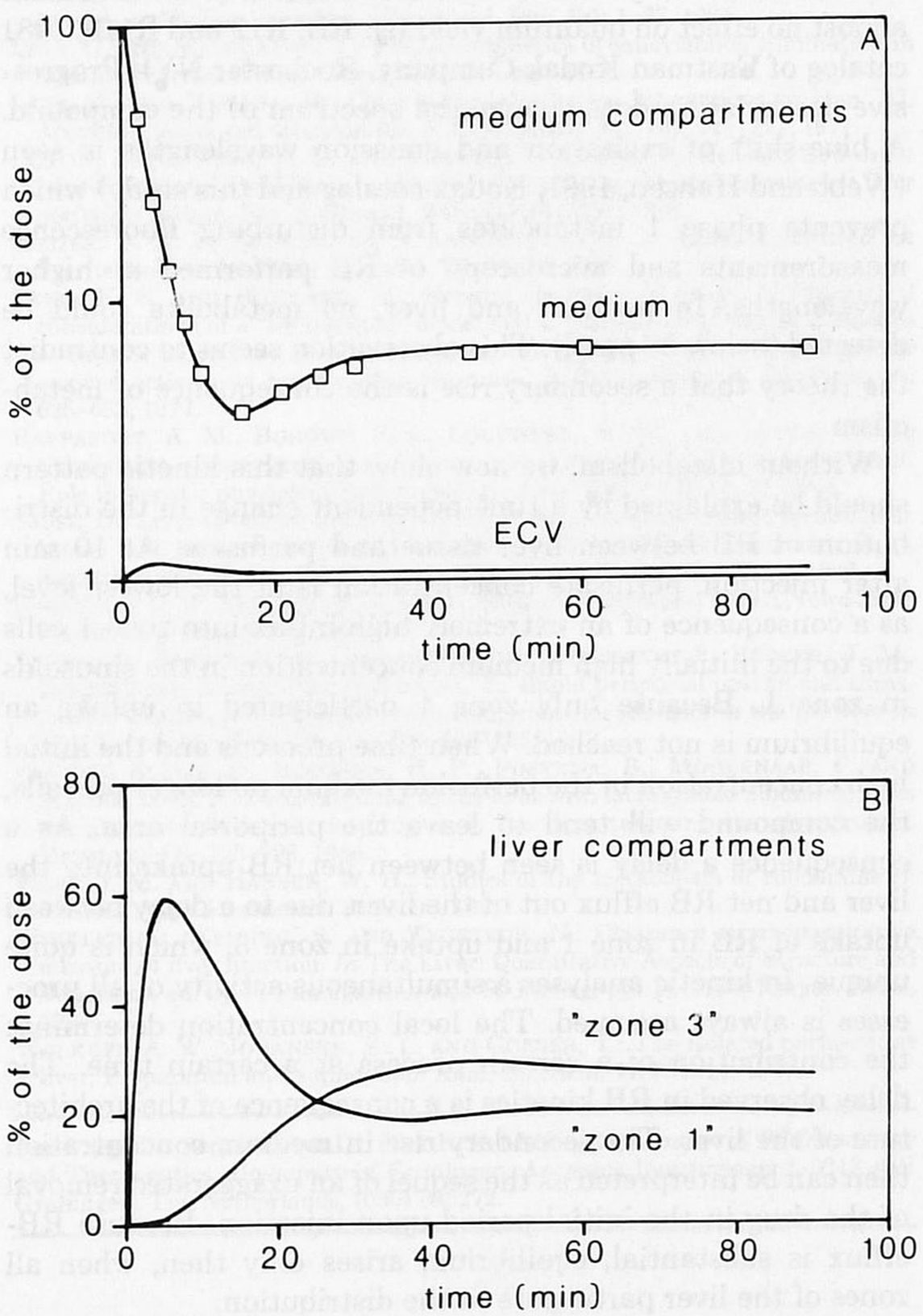

Fig. 3. A, simulated RB-concentration *vs.* time curves from medium (M_{11}, fig. 2) and ECV ($\sum_{i=1}^{10} M_i$); □, measured medium concentrations of RB (*cf.* fig. 1A). B, simulated liver content *vs.* time curves of "zone 1" ($\sum_{i=1}^{3} (L_i+S_i)$) and "zone 3" $\sum_{i=8}^{10} (L_i+S_i)$). $k(M_{11}M_1) = 0.35$; $k(M_iM_{i+1}) = 7$ (i = 1,2,. . .10); $k(M_iL_i) = 17$ (i = 1,2,. . .10); $k(L_iM_i) = 1.30$ (i = 1,2,. . .5); $k(L_iM_i) = 1.77$ (i = 6,7,. . .10); $k(L_iS_i) = 0.23$ (i = 1,2,. . .5); $k(L_iS_i) = 0.37$ (i = 6,7,. . .10); $k(S_iL_i) = 0.32$ (i = 1,2,. . .5); $k(S_iL_i) = 0.18$ (i = 6,7,. . .10); $k(L_iB) = 0.0053$ (i = 1,2,. . .5); $k(L_iB) = 0.0038$ (i = 6,7,. . .10).

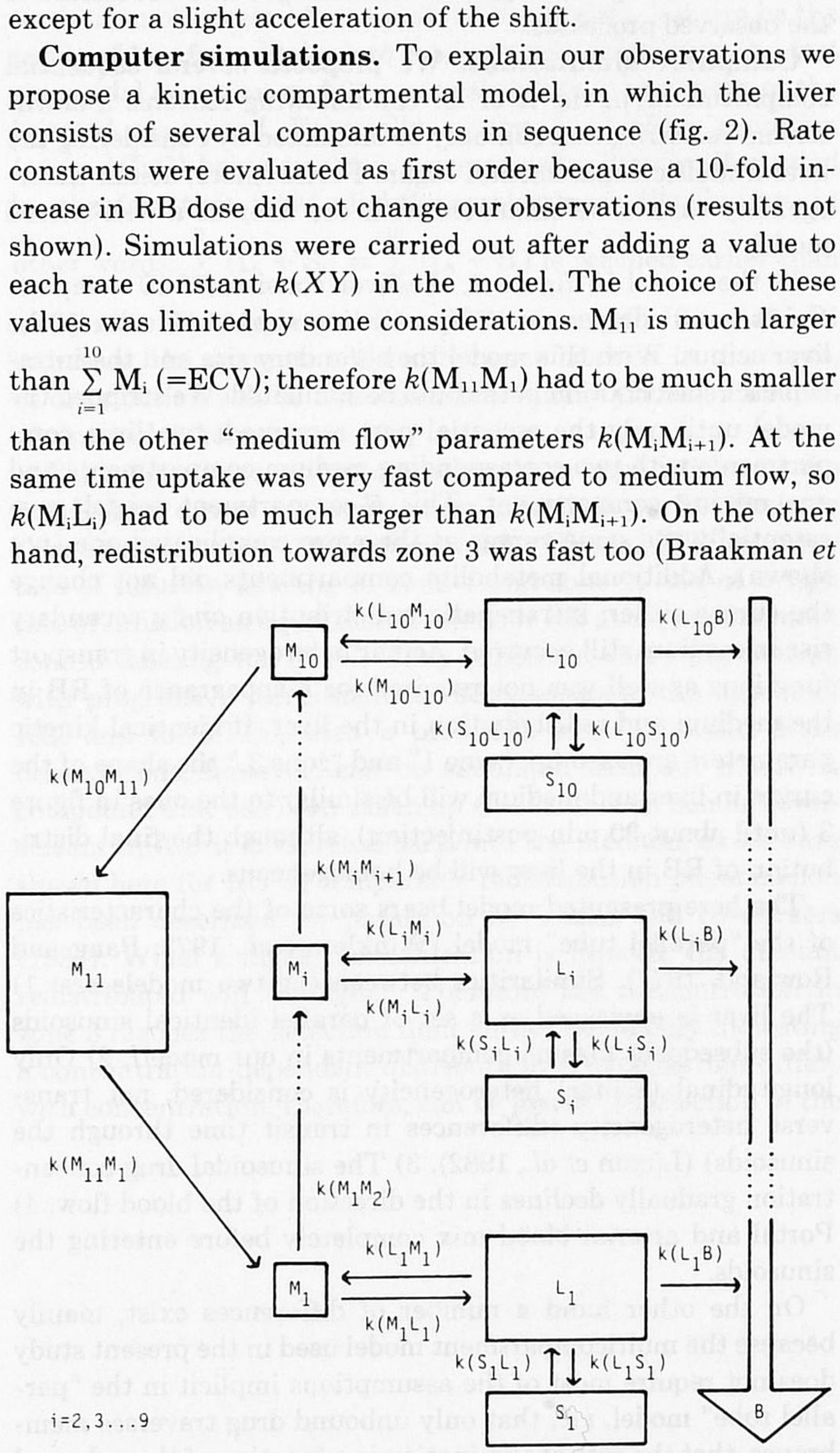

Fig. 2. Compartmental model with 10 sequential liver compartments. M_{11}, central medium reservoir; M_i (i = 1, 2,. . .10), ECV; L_i, liver compartments; S_i, intracellular sequestration sites; B, bile.

the observed zonal fluorescence patterns at different time points (Braakman *et al.*, 1987). The ratio of fluorescence between zone 1 and zone 3 was very high (about 30) in the first period of the experiment, but fell below one (about 0.7) from 25 min on, until the end of the experiment.

Discussion

Kinetic and metabolic pattern of RB. After a flow-limited decline, the perfusate fluorescence rose again in the second part of the curve (fig. 1A). Such a secondary rise is usually associated with metabolic conversions of the injected compound. However, only a small fraction of the dose of RB was metabolized. The unidentified metabolites most probably were the N-demethylated derivatives of RB (Webb and Hansen, 1961). The amount of metabolites was very small, because the intensity of the spots was much lower than that of the glucuronide. Quantum yields were in the same order of magnitude, inasmuch as deethylation (Webb and Hansen, 1961) of RB has almost no effect on quantum yield (*cf.* RB, R19 and R110; 1981 catalog of Eastman Kodak Company, Rochester NY). Progressive deethylation does change the spectrum of the compound. A blue-shift of excitation and emission wavelengths is seen (Webb and Hansen, 1961; Kodak catalog and this study) which prevents phase 1 metabolites from disturbing fluorescence measurements and microscopy of RB performed at higher wavelengths. In medium and liver, no metabolite could be detected (below 20 pmol). This observation seems to contradict the theory that a secondary rise is the consequence of metabolism.

Without metabolism, we now show that this kinetic pattern should be explained by a time-dependent change in the distribution of RB between liver tissue and perfusate. At 10 min after injection, perfusate concentration is at the lowest level, as a consequence of an extremely high influx into zone 1 cells due to the initially high medium concentration in the sinusoids in zone 1. Because only zone 1 participated in uptake an equilibrium is not reached. When time proceeds and the initial high concentration in the perfusion medium no longer prevails, the compound will tend to leave the periportal area. As a consequence a delay is seen between net RB uptake into the liver and net RB efflux out of the liver, due to a delay between uptake of RB in zone 1 and uptake in zone 3, which is quite unique. In kinetic analyses a simultaneous activity of all processes is always assumed. The local concentration determines the contribution of a certain process at a certain time. The delay observed in RB kinetics is a consequence of the architecture of the liver. The secondary rise in medium concentration then can be interpreted as the sequel of an exaggerated removal of the drug in the initial period upon injection. Because RB-efflux is substantial, equilibrium arises only then, when all zones of the liver participate in the distribution.

Influence of SAM. SAM inhibited glucuronidation completely; the RB-glucuronide was no longer detectable. Biliary excretion diminished and was apparently not compensated for by other metabolic pathways (table 1; fig. 1). If SAM would have inhibited biliary transport instead of glucuronidation, a RB-glucuronide would have been found in medium or liver. From the absence of glucuronide we conclude that glucuronidation was largely inhibited, and that SAM had no obvious influence on biliary transport of RB and the remaining metabolites.

The high bile flow in the presence of SAM could well have an osmotic reason (Klaassen and Watkins, 1984); SAM is conjugated with sulfate and glucuronic acid (Levy and Matsuzawa, 1967; Levy and Yamada, 1971) and as such excreted into bile. The higher bile flow did not affect biliary excretion of RB and other metabolites; the amounts excreted were the same with and without SAM.

The shape of the medium concentration *vs.* time curve was not influenced by SAM. The secondary rise was not affected, but the "steady-state" concentration of RB in medium was significantly higher. This is plausible because biliary excretion of RB-equivalents was diminished, which led to a higher liver- and hence a higher medium-concentration of RB. Intrahepatic redistribution of RB was slightly accelerated in the presence of SAM.

From the combined results we concluded that metabolism did not cause the secondary rise in plasma concentration nor the intrahepatic redistribution. We therefore assumed that kinetic modeling would give us more insight into the nature of the observed processes.

Computer simulations. We proposed several sequential compartments in the liver for the following reasons: a downstream redistribution can only be simulated by considering the liver a multicompartmental organ. Furthermore, acinar heterogeneity cannot be simulated with the liver as a well-stirred model.

In the model in figure 2, the liver consists of 10 compartments, each compartment representing about two cells in the liver acinus. With this model the secondary rise and the intrahepatic redistribution both could be simulated. We stripped the model until only the essential part remained: two liver compartments with two corresponding medium compartments and one mixing compartment. This 5-compartment model gave essentially the same curves as the more complicated one (not shown). Additional metabolite compartments did not change the curves either; intrahepatic redistribution *and* a secondary rise in medium still occurred. Acinar heterogeneity in transport functions as well was not required for reappearance of RB in the medium and redistribution in the liver. If identical kinetic parameters are used for "zone 1" and "zone 3," the shape of the curves in liver and medium will be similar to the ones in figure 3 (until about 30 min postinjection), although the final distribution of RB in the liver will be homogeneous.

The here presented model bears some of the characteristics of the "parallel tube" model (Winkler *et al.*, 1973; Pang and Rowland, 1977). Similarities between the two models are: 1) The liver is envisaged as a set of parallel identical sinusoids (the subsequent plasma compartments in our model). 2) Only longitudinal (acinar) heterogeneity is considered, not transverse heterogeneity (differences in transit time through the sinusoids) (Luxon *et al.*, 1982). 3) The sinusoidal drug concentration gradually declines in the direction of the blood flow. 4) Portal and arterial blood mix completely before entering the sinusoids.

On the other hand a number of differences exist, mainly because the multicompartment model used in the present study does not require most of the assumptions implicit in the "parallel tube" model, *i.e.*, that only unbound drug traverses membranes, that the rate of elimination is a function of the unbound drug concentration, that equilibrium exists between drug in blood and drug at the site of excretion, that the rate of distri-

bution in the organ is perfusion limited and that metabolizing enzymes are uniformly distributed in the acinus.

The present model has the advantage that such assumptions *can* be included but are not a prerequisite for the calculations. Essential is that the liver can be modeled by multiple individually defined kinetic compartments with regard to transport and metabolic steps and in that sense can adopt features quite similar to the distributed model (Luxon *et al.*, 1982). In spite of the inherent complexity of the particular model chosen, the calculations are always rapid and easy because they are based on simple (differential) equations.

We concluded from these simulations that a simple model with only five compartments was enough to produce the characteristic medium curve shown in figure 1A, as well as the redistribution as simulated in figure 3B. The curves can be simulated by considering the liver as consisting of at least two sequential compartments instead of one well-stirred compartment. We have shown that a secondary rise in medium concentration is not necessarily caused by metabolism, but can be the result of 1) a fast uptake into the liver, combined with 2) a fast sinusoidal secretion and 3) a very slow biliary excretion of the injected compound. Especially a very slow biliary excretion (every $k(L_iB)$ being small) is a prerequisite. This is illustrated by the fact that SAM slightly accelerates redistribution, in other words: $\sum_{i=1}^{3} (L_i + S_i) = \sum_{i=8}^{10} (L_i + S_i)$ is reached earlier than at 25 min. The opposite will hold true as well: if any $k(L_iB)$ increases, the amounts will be the same later on in the curve, or distribution will be determined by biliary excretion instead of sinusoidal transport.

Taking into account our model can have implications for the conclusions drawn earlier by other investigators. To illustrate this: Gumucio *et al.* (1981) observed for RB and FITC at a low rate of infusion labeling of zone 1 and zone 2, and at a high rate of infusion an equal distribution for RB and a predominant zone 3 labeling for FITC. They explained these phenomena with progressive recruitment of hepatocytes in the uptake of RB, and for FITC, with a preferred zone 3 uptake. Both observations, however, can be simulated with our model. A compound that has been taken up can exhibit an acinar downstream shift if it is excreted back into the medium, as we have shown here for RB. A comparable redistribution phenomenon has been described for parathion by Tsuda and co-workers (1987). When a higher concentration is infused, the amount redistributed will be higher. Therefore the concentration in zone 3 reaches the detection limit earlier, seemingly indicating a concentration-dependent distribution. A pattern that differs with concentration, therefore, can be merely a reflection of the distribution process along with time. Hence a zonal distribution should always be determined at more than one timepoint.

Acknowledgments

It is a pleasure to acknowledge Professor Dr. Gerard J. Mulder for his "metabolic advice," Ferdie Rombout for his assistance with the computer simulation studies and Dr. Peter van der Sluijs for many valuable discussions. Angelie Braakman is thanked for secretarial assistance.

References

BOUTIN, J. A., THOMASSIN, J., SIEST, G. AND BATT, A.-M.: Inhibition studies of microsomal UDP-glucuronosyl transferase activities by furosemide and salicylamide. Pharmacol. Res. Commun. **16:** 227–241, 1984.

BRAAKMAN, I., GROOTHUIS, G. M. M. AND MEIJER, D. K. F.: Acinar redistribution and heterogeneity in transport of the organic cation rhodamine B in rat liver. Hepatology **7:** 849–855, 1987.

GORES, G. J., KOST, L. J. AND LARUSSO, N. F.: The isolated perfused rat liver: Conceptual and practical considerations. Hepatology **6:** 511–517, 1986.

GUMUCIO, J. J., MILLER, D. L., KRAUS, M. D. AND ZANOLLI, C. C.: Transport of fluorescent compounds into hepatocytes and the resultant zonal labeling of the hepatic acinus in the rat. Gastroenterology **80:** 639–646, 1981.

HOWELL, S. R., HAZELTON, G. A. AND KLAASSEN, C. D.: Depletion of hepatic UDP-glucuronic acid by drugs that are glucuronidated. J. Pharmacol. Exp. Ther. **236:** 610–614, 1986.

KLAASSEN, C. D. AND WATKINS, III: J. B.: Mechanisms of bile formation, hepatic uptake, and biliary excretion. Pharmacol. Rev. **36:** 1–67, 1984.

LEVY, G. AND MATSUZAWA, T.: Pharmacokinetics of salicylamide elimination in man. J. Pharmacol. Exp. Ther. **156:** 285–293, 1967.

LEVY, G. AND YAMADA, H.: Drug biotransformation interactions in man. III. Acetaminophen and salicylamide. J. Pharmaceut. Sci. **60:** 215–221, 1971.

LUXON, B. A., KING, P. D. AND FORKER, E. L.: How to measure first-order hepatic transfer coefficients by distributed modeling of a recirculating rat liver perfusion system. Am. J. Physiol. **243:** G518–G531, 1982.

MEIJER, D. K. F., KEULEMANS, K. AND MULDER, G. J.: Isolated perfused rat liver technique. Methods Enzymol. **77:** 81–94, 1981.

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 cell binding, and the hepatocellular enzyme activity on hepatic drug clearance. J. Pharmacokinet. Biopharm. **5:** 625–653, 1977.

RAPPAPORT, A. M., BOROWY, Z. J., LOUGHEED, W. M. AND LOTTO, W. N.: Subdivision of hexagonal liver lobules into a structural and functional unit. Role in hepatic physiology and pathology. Anat. Rec. **119:** 11–27, 1954.

SASSE, D.: Liver structure and innervation. *In* Regulation of hepatic metabolism. Intra- and intercellular compartmentation, ed. by R. G. Thurman, F. C. Kauffman and K. Jungermann, pp. 3–25, Plenum Press, New York, 1986.

SNEDECOR, G. W. AND COCHRAN, W. G.: Statistical Methods, 7th ed., Iowa State University Press, Ames, 1980.

TSUDA, S., SHERMAN, W., ROSENBERG, A., TIMOSZYK, J., BECKER, J. M., KEADTISUKE, S. AND NAKATSUGAWA, T.: Rapid periportal uptake and translobular migration of parathion with concurrent metabolism in the rat liver *in vivo*. Pestic. Biochem. Physiol. **28:** 201–215, 1987.

VAN DER SLUIJS, P., BOOTSMA, H. P., POSTEMA, B., MOOLENAAR, F. AND MEIJER, D. K. F.: Drug targeting to the liver with lactosylated albumins: Does the glycoprotein target the drug or is the drug targeting the glycoprotein. Hepatology **6:** 723–728, 1986.

WEBB, J. M. AND HANSEN, W. H.: Studies of the metabolism of rhodamine B. Toxicol. Appl. Pharmacol. **3:** 86–95, 1961.

WINKLER, K., KEIDING, S. AND TYGSTRUP, N.: Clearance as a quantitative measure of liver function. *In* The Liver: Quantitative Aspects of Structure and Functions, ed. by P. Paumgartner and R. Preisig, pp. 144–155, Karger, Basel, 1973.

WOLKOFF, A. W., JOHANSEN, K. L. AND GOESER, T.: The isolated perfused rat liver: Preparation and application. Anal. Biochem. **167:** 1–14, 1987.

Send reprint requests to: Dr. Dirk K. F. Meijer, Department of Pharmacology and Therapeutics, University of Groningen, Antonius Deusinglaan 2, 9713 AW Groningen, The Netherlands, (050) 633272.