Distribution Kinetics of Salicylic Acid in the Isolated Perfused Rat Liver Assessed Using Moment Analysis and the Two-Compartment Axial Dispersion Model

Ziad Hussein,[1,2] Andrew J. McLachlan,[1,3] and Malcolm Rowland[1,4]

Received February 25, 1994; accepted April 19, 1994

The distribution kinetics of salicylic acid in the single-pass isolated perfused rat liver has been investigated under varying conditions of perfusate flow (15 to 30 ml min^{-1}) and of salicylate perfusate concentration (0, 100, 200 mg l^{-1}) using statistical moment analysis and the two-compartment axial dispersion model. Salicylic acid was not metabolised during the experiment. The perfusate did not contain binding protein. As flow rate was increased, the maximum fraction output per second ($f(t)_{max}$) increased and the mean transit time (MTT_H) decreased, while t_{max} became shorter for both tritiated water and ^{14}C-salicylic acid. Increasing the salicylate perfusate concentration profoundly affected the frequency outflow profile of ^{14}C-salicylic acid, but not that of tritiated water. The one-compartment axial dispersion model adequately described the frequency outflow profile for tritiated water, whereas the two-compartment form, which incorporates a cellular permeability barrier, provided a better description of the ^{14}C-salicylic acid outflow data. The estimated two-compartment axial dispersion model parameters for ^{14}C-salicylic acid, D_N, the dispersion number (0.08 ± 0.03), k_{12}, the influx rate constant (0.56 ± 0.04 sec^{-1}) and k_{21}, the efflux rate constant (0.095 ± 0.01 sec^{-1}) were independent of perfusate flow rate. The *in situ* permeability-surface area product for ^{14}C-salicylic acid (4.6 ± 0.7 ml $min^{-1}g^{-1}$ liver) was in good agreement with literature estimates obtained from *in vitro* hepatocyte experiments, suggesting that the permeability barrier is at the hepatocyte membrane. Whereas D_N and k_{12} were uninfluenced by, k_{21} displayed a positive correlation with, salicylate perfusate concentration. This correlation was most likely due to decreased intracellular salicylate binding.

KEY WORDS: salicylic acid; dispersion model; hepatic disposition; tissue binding.

INTRODUCTION

Investigations into the mechanism by which drugs distribute into and throughout the liver provide important insights into the pharmacokinetics of a drug. The rate and extent of distribution and elimination of a solute in the liver is influenced by a number of factors; hepatic perfusate flow rate, intrinsic clearance, the extent to which a solute is bound in the perfusate, the tissue-perfusate partition coefficient and the membrane permeability of a solute (1).

Previous studies with lipophilic compounds that bind extensively to plasma proteins, show that variation in the extent of protein binding in the perfusate profoundly affects the distribution and elimination of solutes in the liver (2–5). The rate and extent of distribution of highly bound solutes into hepatic tissue is decreased as the extent of binding in the perfusate is increased. These data are consistent with the concept that the observed effects are due to a lowering of the diffusion gradient for distribution and elimination.

The isolated perfused rat liver (IPRL) preparation is an ideal experimental system that has been widely used to investigate the kinetics of hepatic distribution and elimination for many solutes. Impulse-response experiments, which employ the IPRL preparation in single-pass mode, provide an insight into the processes that act on an injected solute during passage through the liver. A number of models have been proposed to predict and describe kinetic events in the liver (1,6). Of these models, the axial dispersion model best describes outflow data obtained from impulse-response (6,7) and steady-state (8) experiments using the IPRL. The original one-compartment form (7) of the axial dispersion model assumes spontaneous radial distribution of solutes between perfusate and cells. In practice the two-compartment form (9), which accommodates non-instantaneous distribution, is needed to successfully describe the distribution kinetics of several solutes during impulse-response experiments, particularly in the presence of the binding protein (3–5).

The disposition kinetics of salicylic acid has been extensively studied in animals (10–12) and humans (13). Salicylic acid displays low permeability across the hepatocyte membrane *in vitro* (14) and binds within the liver (11). As such it provides an opportunity to examine the factors affecting the hepatic distribution kinetics of relatively poorly permeable compounds. The aims of the present study are to analyse and attempt to model successfully the influence of variations in perfusion flow rate and hepatic tissue binding on the distribution kinetics of salicylic acid in the IPRL using statistical moment analysis and the two-compartment axial dispersion model.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats weighing between 350 g and 400 g, fed on a normal laboratory diet and with free access to water, were used in the liver perfusion experiments.

Materials. All reagents were of analytical grade. Tritiated water (5 Ci ml^{-1}) and ^{14}C-labelled salicylic acid (53.8 mCi $mmol^{-1}$) were obtained from Amersham (UK) and were used without further purification. Sodium salicylate was purchased from BDH (UK).

Liver Perfusion Experiments. The single-pass isolated perfused *in situ* liver preparation was the same as that de-

[1] Department of Pharmacy, University of Manchester, Manchester, M13 9PL, UK.

[2] present address: Department of Clinical Pharmacology, The Wellcome Foundation, Langley Court, Beckenham, Kent, BR3 3BS, UK.

[3] present address: Department of Clinical Pharmacology and Toxicology, St Vincent's Hospital, NSW 2010, Australia.

[4] corresponding author Prof Malcolm Rowland Department of Pharmacy Coupland Building III University of Manchester Oxford Rd Manchester M13 9PL United Kingdom Telephone -61-275-2348 Facsimile -61-273-8196

0724-8741/94/0900-1337$07.00/0

scribed previously (3–5). All experiments were conducted at 37°C. Under intraperitoneal anaesthesia, with 50 mg/kg sodium pentobarbital (Sagital), the bile duct was cannulated with PE-10 tubing. After rapid cannulation and ligation of the portal vein with a 2.0 mm I.D. cannula, the liver was perfused with Krebs-Ringer bicarbonate buffer (pH 7.4), containing 300 mg/dl glucose and saturated with humidified and warmed O_2:CO_2 (95%:5%), at a variety of flow rates (described below). The superior vena cava was cannulated through the right atrium with 2.0 mm I.D. cannula without interruption to the portal perfusion. The inferior vena cava was ligated above the renal vein.

Study I: Effect of perfusate flow rate on the distribution kinetics of ^{14}C-salicylic acid. After a stabilization period of 15 min at a perfusate flow rate of 15 ml min^{-1}, 50 μl of tritiated water (0.5 μCi), as a marker for total water volume, and 25 μg of ^{14}C-salicylic acid (0.25 μCi) were injected simultaneously as a bolus into the portal vein. The total perfused effluent from the hepatic vein was collected at 1.5 sec intervals for 85.5 seconds using an automated turntable. The flow rate was increased to 20 ml min^{-1} and the preparation was allowed to stabilise for a 10 min period after which time a second bolus injection was administered as described above. Similarly, a third, fourth and fifth injection was performed, after a 10 min stabilisation at 25, 30 and 15 ml min^{-1}, respectively. If a reproducible frequency outflow profile was obtained after the first and fifth bolus injections (both at 15 ml min^{-1}) the liver perfusion preparation was judged to be viable during the study period and the experimental results adopted. Liver viability was also routinely assessed by monitoring bile flow, perfusate recovery and by macroscopic appearance. Liver weight was determined at the end of the experiment.

Study II: The effect of varying concentrations of salicylate in the perfusate on the distribution kinetics of ^{14}C-salicylic acid. In the first phase (Phase A) of this experiment, two bolus injections of tritiated water and ^{14}C-salicylic acid were performed with the same rat liver at a flow rate of 20 and 25 ml min^{-1}, respectively, as described for Study I. In the second phase (Phase B) of this experiment the original perfusate was replaced with fresh perfusion media containing 100 mg l^{-1} of salicylic acid. The preparation was allowed to equilibrate with the new perfusate for 15 min, to ensure that steady-state conditions were achieved, at a flow rate of 20 ml min^{-1}. A bolus injection of both solutes was performed and samples collected as described above. The flow rate was then increased to 25 ml min^{-1} after a further 15 min another injection was administered. The third phase (Phase C) of this study, the experiment was repeated with a perfusate containing 200 mg l^{-1} salicylic acid using the same liver.

Sample Analysis. The concentrations of tritiated water and ^{14}C-labelled salicylic acid in samples of perfusion effluent were measured by radiochemical analysis. Samples (0.3 ml) were counted after addition of 5 ml of scintillant (LKB Optiphase, Hisasfe II) using a dual quench curve for ^{3}H and ^{14}C determined with a LKB Rackbeta Liquid Scintillation Counter. Samples 1 to 20 were counted individually (300 μl), sample 21 to 40 were counted as aliquots (150 μl) of consecutive pairs and samples 41 to 56 as aliquots (75 μl) of four consecutive pairs. Perfusate concentrations of unlabelled salicylic acid obtained from preliminary steady-state perfusion experiments (Study II) were analysed using the HPLC method of Cham *et al* (15).

DATA ANALYSIS

The frequency output (f(t)), fractional output per second, of the injected material at the midpoint time of the collection interval was calculated using the following equation,

$$f(t) = \frac{C(t).Q}{D} \tag{1}$$

where C(t) is the concentration of radioactivity for each solute, Q is the perfusate flow rate and D is the injected dose. The maximum f(t) value, $f(t)_{max}$, and the time at which it occurred, t_{max}, were observed values.

Statistical Moment Analysis. The area under the outflow concentration-time profile (AUC) and the mean transit time (MTT) of the solute in the whole experimental system (hepatic and non-hepatic regions) were calculated using the following equations,

$$AUC = \int_0^\infty C(t)\, dt \tag{2}$$

$$MTT = \frac{\int_0^\infty t \,.\, C(t)\, dt}{AUC} \tag{3}$$

These equations assume that a solute is not eliminated and Q is constant throughout. Area was estimated using the linear trapezoidal rule with a log-linear extrapolation to obtain the AUC from the last observation to ∞. The AUC for ^{14}C-salicylic acid was expressed as a ratio of the AUC of the non-eliminated tritiated water to gain an estimate of recovery. The mean transit time of solute in the non-hepatic regions of the experimental system (MTT_{NH}) was calculated in preliminary experiments where the inflow and outflow cannuli were directly connected. MTT_H, the mean transit time of solute within the liver, was obtained by subtracting MTT_{NH} from the observed MTT for the entire experimental system. The volume of distribution of the solute in the liver (V_H) was obtained using Equation 4.

$$V_H = Q \cdot MTT_H \tag{4}$$

Application of the axial dispersion model to characterise solute outflow profile. The approach used to characterise the frequency outflow versus time data of a solute has been described in previous publications (3–5). Assuming linear and time invariant conditions, the frequency output ($f(t)_{+L}$) of a solute injected into the single-pass isolated perfused rat liver preparation is the convolution of a series of successive weighting functions, each describing a region within the experimental system. This can be represented by the following equation,

$$f(t)_{+L} = x(t) * w(t)_H * w(t)_{NH} \tag{5}$$

where the * symbol denotes the convolution operation, x(t) is a weighting function that describes the input form of the dose and, $w(t)_H$ and $w(t)_{NH}$ are the weighting functions that describe the spread of a solute on passage through the he-

patic and non-hepatic regions (catheters, tubing, collection device) of the experimental system, respectively.

In the Laplace domain the relationship depicted in Equation 5 simplifies to a multiplication procedure,

$$f(s)_{+L} = x(s) \cdot w(s)_H \cdot w(s)_{NH} \quad (6)$$

where s is the Laplace operator. It is possible to investigate the transfer function of the non-hepatic regions ($w(s)_{NH}$) using an experimental system which does not include the liver, that is, where the inflow and outflow cannuli are connected directly. The Laplace of the frequency outflow from this experimental system ($f(s)_{-L}$) is given as,

$$f(s)_{-L} = x(s) \cdot w(s)_{NH} \quad (7)$$

If the dose is delivered in the form of a rapid bolus (unit impulse) x(s) is equal to one, and equations 6 and 7 simplify further.

The transfer function for distribution of solutes in the liver can be described using the axial dispersion model (6,7). In the present analysis mixed (type I) boundary conditions were assumed (7,9). For a non-eliminated solute whose radial distribution into hepatic tissue is instantaneous the transfer function for the liver during a single pass can be adequately described using the one-compartment axial dispersion model (7), so that,

$$w(s)_H = \exp\left[\frac{1 - \sqrt{1 + \frac{4\,D_N\,V_H\,s}{Q}}}{2\,D_N}\right] \quad (8)$$

where D_N is the hepatic dispersion number, a measure of axial spreading of a solute in the liver.

In the case where a permeability barrier exists within the liver, such that radial distribution of a non-eliminated solute into hepatic tissue is not instantaneous, the hepatic transfer function is described by the two-compartment axial dispersion model (3–5,9),

$$w(s)_H = \exp\left[\frac{1 - \sqrt{1 + \frac{4\,D_N\,V_B}{Q}\left[k_{12} + s - \frac{k_{12}\,k_{21}}{s + k_{21}}\right]}}{2\,D_N}\right] \quad (9)$$

where V_B is the volume of the central compartment (which physiologically represents the combined volumes of the vascular and Disse spaces in the liver) and the transfer rate constants, k_{12} and k_{21} represent the influx and efflux first-order rate constants across the hepatic cellular membrane, respectively. The influx and efflux rate constants can be further defined as,

$$k_{12} = \frac{PS\,fu}{V_B} \quad (10)$$

$$k_{21} = \frac{PS\,fu_c}{V_c} \quad (11)$$

where PS is the permeability-surface area product (with units of volume per time) of the hepatocyte membrane to the solute, fu and fu_c are the unbound fractions of the solute in the perfusate and cell, respectively, and V_c is the aqueous volume of the cellular space.

The transfer function of the frequency outflow curve obtained from the experimental system without the liver present can be described using a rearranged version of Equation 8,

$$w(s)_{NH} = \exp\left[\frac{1 - \sqrt{1 + 4.D_{N,NH} \cdot MTT_{NH} \cdot s}}{2 \cdot D_{N,NH}}\right] \quad (12)$$

where $D_{N,NH}$ is the dispersion number of the solute in the non-hepatic perfusion system (3–5) and MTT_{NH} has been defined previously.

Fitting Procedure. Frequency outflow versus time data were modelled with the time domain solutions of the appropriate transfer functions using a numerical inversion program (MULTI-FILT version 3.4) (9). The damping Gauss Newton minimisation algorithm was employed and the data were equally weighted in the non-linear least squares analysis.

The axial dispersion model parameters for the non-hepatic region of the experimental system have been determined in previous experiments in our laboratory (3–5). $D_{N,NH}$ was reported to be 0.04 and the value for MTT_{NH} at each flow rate was calculated using the relationship, $MTT_{NH} = V_{NH}/Q$, where V_{NH}, the volume of the non-hepatic region of the experimental system, was calculated to be 1.125 ml. These values were assumed to apply equally to tritiated water and ^{14}C-salicylic acid.

Frequency outflow data for tritiated water and ^{14}C-salicylic acid was modelled using a one-compartment (Equation 8) and a two-compartment axial dispersion model (Equation 9), respectively. V_B was assigned *a priori* as 15% of liver weight (previously validated; 3–5) and remained fixed in the modelling procedure. Data for both solutes obtained in Study I were analysed individually.

In Study II ^{14}C-salicylic acid frequency outflow data, obtained from the same liver at different concentrations of salicylate in the perfusate (Phases A, B and C), were analysed simultaneously. The full model used to describe events in the liver has 7 parameters (D_N, $k_{12,A}$, $k_{21,A}$, $k_{12,B}$, $k_{21,B}$, $k_{12,C}$ and $k_{21,C}$). In the second reduced model (Reduced model I) the same value of the influx first-order rate constant (k_{12}) was assumed to apply to all treatment phases (i.e., $k_{12,A} = k_{12,B} = k_{12,C}$). The model reduction is based on the assumption that k_{12} is independent of concentration of salicylate in the perfusate. Reduced model I has 5 parameters (D_N, k_{12}, $k_{21,A}$, $k_{21,B}$ and $k_{21,C}$). In a further reduction (Reduced model II) the value of D_N was also fixed to that obtained for the simultaneous analysis of tritiated water and ^{14}C-salicylic acid frequency outflow data. Reduced model II has only 4 parameters (k_{12}, $k_{21,A}$, $k_{21,B}$ and $k_{21,C}$). Full and reduced models were compared using the F-ratio test based on residual weighted sum of squares (16). The calculated F values were compared to the critical value at the 5% level of significance.

RESULTS

Figure 1 shows frequency outflow versus time plots of tritiated water and ^{14}C-salicylic acid from a representative liver at different flow rates. In general, after bolus injection

of ^{14}C-salicylic acid into the hepatic portal vein the frequency outflow curve displays a characteristic sharp peak that elutes from the experimental system over approximately 15 seconds, followed by a slower eluting flat tail (Figure 1B). In Table I are listed the mean $f(t)_{max}$ and t_{max} values (n = 4) obtained under different perfusate flow rates. An increase in perfusate flow rate resulted in notable changes in the shape of the frequency outflow profile for both solutes. The $f(t)_{max}$ for tritiated water displayed a two-fold increase and for ^{14}C-salicylic acid a three-fold increase as flow rate was increased, from 15 to 30 ml min^{-1}. For both solutes the t_{max} became shorter with increasing perfusate flow rate.

Figure 2 shows the effect of increasing the concentration of salicylate in the perfusate on the frequency outflow profiles of tritiated water and ^{14}C-salicylic acid in the same liver perfused at 20 ml min^{-1}. The shape of the profile of tritiated water was unaffected by the presence of salicylate in the perfusate. This is confirmed by the mean $f(t)_{max}$ and t_{max} data (n = 2) obtained at two flow rates (20 and 25 ml min^{-1}) presented in Table II. In contrast, the increasing concentration of salicylate in the perfusate had a profound effect in the shape of the frequency outflow profile of ^{14}C-salicylic acid. At both flow rates the value of $f(t)_{max}$ for ^{14}C-salicylic acid displayed a concentration-dependent increase while t_{max} remained largely unchanged (Table II).

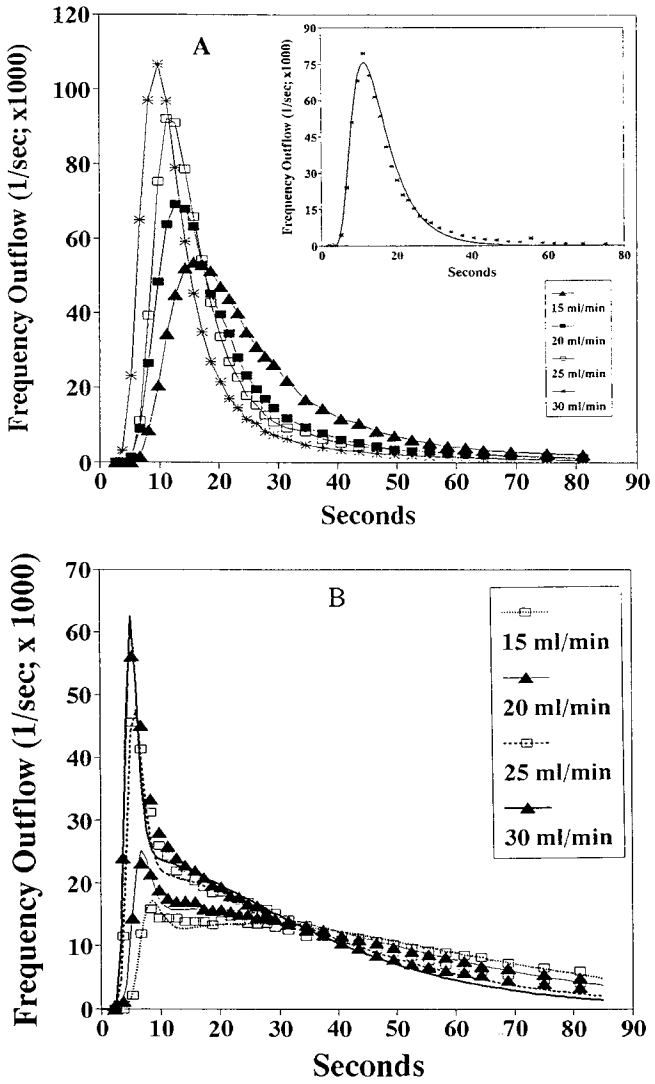

Figure 1: Typical frequency outflow versus time profiles of **(A) tritiated water** (observed data only, the inset diagram shows a typical fit of the one-compartment dispersion model to tritiated water data at 30 ml min^{-1}) and **(B) ^{14}C-salicylic acid** (observed and fitted data using the two-compartment dispersion model) in the isolated perfused rat liver at different perfusate flow rates.

STATISTICAL MOMENT ANALYSIS

Tables I and II present the results of statistical moment analysis of the outflow data for tritiated water and ^{14}C-salicylic acid in Study I and II, respectively. The dose-normalised $AUC_{0-\infty}$ of ^{14}C-salicylic acid is presented as a ratio of the $AUC_{0-\infty}$ of the co-injected non-eliminated reference, tritiated water. The normalised $AUC_{0-\infty}$ ratio was essentially one, indicating total recovery of the injected dose of ^{14}C-salicylic acid. The agreement of the normalised $AUC_{0-\infty}$ ratios across each of the study phases indicates that perfusate flow rate and salicylate concentration did not alter ^{14}C-salicylic acid recovery.

The mean transit time for both solutes, corrected for transit time in the non-hepatic regions of the experimental system, displayed a statistically significant ($p < 0.001$) and characteristic decline with an increase in perfusate flow rate in the absence of salicylate in the perfusate. The volume of distribution for both solutes showed a slight increase with increasing perfusate flow rate from 15 to 30 ml min^{-1}, corresponding to 1.18 to 2.34 ml min^{-1} g^{-1} liver, that reached statistical significance for ^{14}C-salicylic acid (1.22 to 1.59 ml g^{-1}; $p = 0.006$) but not for tritiated water (0.51 to 0.66 ml g^{-1} liver; $p = 0.08$) (Table I). The apparent volume of distribution of ^{14}C-salicylic acid was more than twice that observed for tritiated water.

The MTT_H of tritiated water in the rat liver was not influenced by the presence of salicylate in the perfusate (Table II). In contrast, the presence of salicylate in the perfusate resulted in a concentration dependent decrease in both MTT_H and V_H of ^{14}C-salicylic acid at both of the study flow rates.

APPLICATION OF THE AXIAL DISPERSION MODEL

Study I: Effect of flow rate on ^{14}C-salicylic acid distribution kinetics in the rat liver

Figure 1B shows the fit of the two-compartment dispersion model equation to the frequency outflow profile of ^{14}C-salicylic acid at perfusate flow rates of 15, 20, 25 and 30 ml min^{-1}.

The dispersion model parameter estimates for tritiated water and ^{14}C-salicylic acid at different flow rates are presented in Table III. The D_N for tritiated water (typical fit displayed in Figure 1A inset), 0.19 ± 0.04 (Table III), was not influenced by the increase in perfusate flow rate ($p = 0.31$) while V_H displayed a tendency to increase slightly with increasing flow rate which just reached statistical significance ($p = 0.04$). The two-compartment dispersion model parameters (D_N, k_{12} and k_{21}) for ^{14}C-salicylic acid were estimated with a high degree of precision (coefficient of variation of less than 10%). Analysis of variance indicates that all param-

Table I. Mean (±SD) observational and statistical moments analysis parameters for tritiated water and ^{14}C-salicylic acid in the isolated perfused rat liver (mean weight 13.2 ± 0.9 g, n = 4) at four different perfusate flow rates.

Parameter	TRITIATED WATER				^{14}C-SALICYLIC ACID			
Flow rate (ml min^{-1})	15	20	25	30	15	20	25	30
$f(t)_{max}$[a] ($\times 10^3$)	51 ± 6	69 ± 5	77 ± 12	91 ± 17	16 ± 4	26 ± 6	33 ± 10	45 ± 11
t_{max} (sec)	18.0 ± 2.9	14.3 ± 1.2	13.1 ± 1.4	11.2 ± 1.5	8.3 ± 0.1	7.1 ± 0.8	6.8 ± 1.2	5.8 ± 0.9
$AUC_{0-\infty}$ ratio					0.93 ± 0.07	0.97 ± 0.12	0.95 ± 0.04	1.00 ± 0.10
MTT_H[c] (sec)	26.9 ± 1.6	21.3 ± 0.8	20.0 ± 0.3	17.5 ± 1.9	64.2 ± 2.9	53.1 ± 3.7	47.3 ± 6.1	41.7 ± 5.9
V_H (ml g^{-1})	0.51 ± 0.04	0.54 ± 0.03	0.63 ± 0.03	0.66 ± 0.06	1.22 ± 0.02	1.35 ± 0.09	1.50 ± 0.19	1.59 ± 0.25

[a] f(t): outflow fraction per second
[b] ratio of $AUC_{salicylate}/AUC_{tritiated\ water}$
[c] corrected for MTT_{NH}

eters appeared to be independent of perfusate flow rate ($p = 0.31$, $p = 0.58$, $p = 0.082$ for D_N, k_{12} and k_{21}, respectively). The mean (±SD) values of D_N, k_{12} and k_{21} were 0.08 ± 0.03, 0.56 ± 0.04 sec^{-1} and 0.095 ± 0.01 sec^{-1}, respectively. An estimate of the apparent tissue-perfusate partition coefficient (Kp′) was obtained from the ratio of the efflux and influx rate constants (k_{12}/k_{21}) (17). This was essentially constant across the range of perfusate flow rates at 6.1 ± 0.7.

Study II: Effect of perfusate salicylate concentration on the distribution kinetics of ^{14}C-salicylic acid in the rat liver

Table IV lists the one- and two-compartment dispersion model parameter estimates for tritiated water and ^{14}C-salicylic acid, respectively, at different salicylate concentrations in the perfusate. The one-compartment dispersion model parameters for tritiated water (D_N = 0.17 ± 0.03 and 0.15 ± 0.04; V_H = 5.9 ± 0.3 and 5.3 ± 0.5 ml, at 20 and 25 ml min^{-1}, respectively) were not influenced by the presence of salicylate in the perfusate (Table IV).

A comparison of full and reduced models indicated that the frequency outflow profile of ^{14}C-salicylic acid was well described by Reduced model I ($p < 10^{-6}$) in which the first-order influx rate constant (k_{12}) is independent of salicylate concentration in the perfusate (i.e., $k_{12,A} = k_{12,B} = k_{12,C}$). The further constraint of fixing D_N for ^{14}C-salicylic acid to be the same as that observed for tritiated water (Reduced model II) could not adequately describe the observed outflow profiles.

Figure 2B shows the two-compartment dispersion model fits for ^{14}C-salicylic acid under the three study conditions at 20 ml min^{-1}. This model was capable of adequately describing the change in the shape of the frequency outflow profile when the concentration of salicylate in the perfusate was increased. However, the model tended to underestimate the terminal portion of the outflow curve for ^{14}C-salicylic acid at the highest perfusate concentration of unlabelled drug (200 mg l^{-1}). This was observed in all Phase C (200 mg l^{-1} salicylate in perfusate) data sets at both flow rates and appeared to be independent of the weighting scheme employed.

For ^{14}C-salicylic acid, the values of D_N (0.08 and 0.09) and k_{12} (0.45 and 0.45 sec^{-1}) (Table IV), assumed to be independent of salicylate perfusate concentration, were in good agreement with the dispersion model parameters found in Study I (shown in Table III). An increase in the concentration of salicylate in the perfusate had a profound effect on the value of the first-order efflux rate constant, k_{21}. This parameter displayed a concentration dependence at both flow rates studied. Thus, the estimated value of the Kp′ was dramatically reduced (6.3 to 3.3 and 5.7 to 1.9, at 20 and 25 ml min^{-1}, respectively) as the concentration of salicylate in the perfusate was increased.

Estimation of the throughput component of the ^{14}C-salicylic acid outflow profile

In addition to D_N, the shape of the frequency outflow profile for ^{14}C-salicylic acid is influenced by two processes; hepatocyte permeability and intra-hepatocyte binding. The rapidly eluting peak of the ^{14}C-salicylic acid profile represents the throughput component, the fraction of the total outflow that does not leave the vascular space. That is, material that has not had time to move out of the vascular space on its passage through the liver, primarily due to poor cellular permeability. Conversely, the more slowly eluting fraction of the output, corresponding to the 'returning component' (18), represents ^{14}C-salicylic acid that has entered cells and returned to the vascular compartment impeded by intracellular binding and limited permeability. Using the dispersion number (D_N) and first-order influx rate constant (k_{12}) parameters presented in Tables III and IV it is possible to estimate the throughput components for ^{14}C-salicylic acid. The throughput component is calculated as the percentage difference in AUC of the outflow profile obtained by performing simulations firstly using typical values for k_{21} (Table IV) and then by setting the efflux rate constant (k_{21}) to zero (i.e., no return of ^{14}C-salicylic acid from the cellular space) (18). The throughput component for ^{14}C-salicylic acid in the absence of salicylate in the perfusate increased with increasing flow rate (3.7%, 7.9%, 12.2% and 11.5% at 15, 20, 25 and 30 ml min^{-1}, respectively). These simulations illustrate the

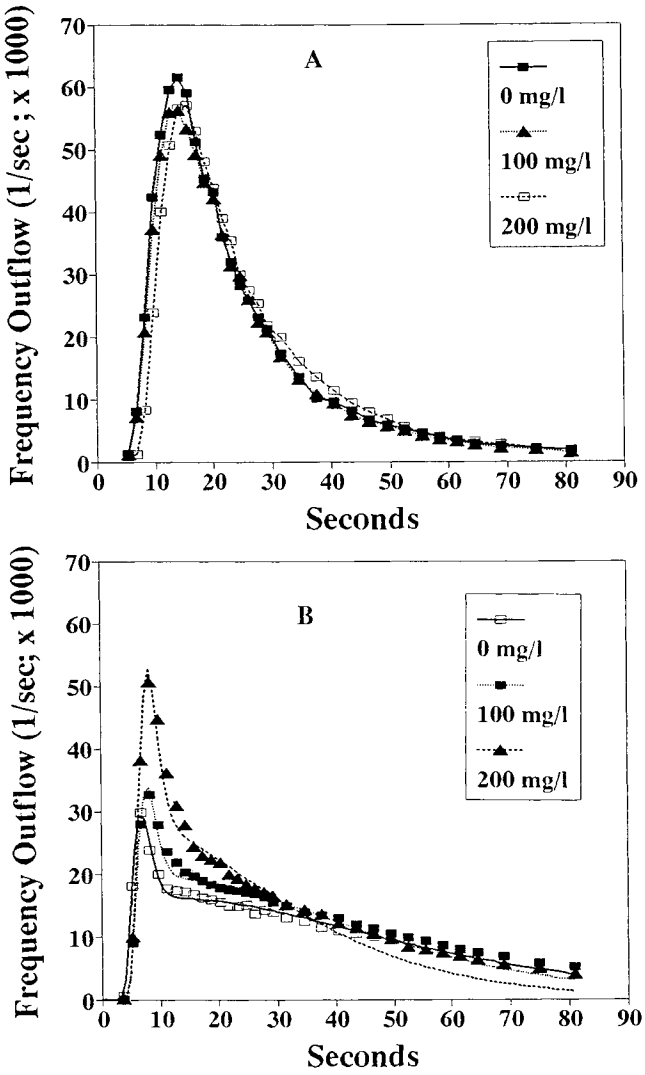

Figure 2: Typical frequency outflow versus time profiles of (A) **tritiated water** (observed data only) and (B) **^{14}C-salicylic acid** (observed and fitted data using the two-compartment dispersion model) in the isolated perfused rat liver at different concentrations of salicylate in the perfusate at 20 ml min^{-1}.

relative effect of alteration of perfusate flow rate on the outflow profile for ^{14}C-salicylic acid.

DISCUSSION

The single-pass *in situ* isolated perfused rat liver has been well documented as an ideal preparation for the investigation of the rate and extent of hepatic tissue uptake and distribution of solutes (3–6). The interest of the present study was to examine the distribution kinetics of salicylic acid in the IPRL preparation because this compound is known to undergo significant binding in hepatic tissue. The presence of high concentrations of unlabelled salicylate and the absence of albumin, salicylic acid's binding protein, in the perfusate has made it possible to examine, for the first time, the effect of an alteration in the hepatic tissue binding

Table II. Mean observational and statistical moment analysis parameters for tritiated water and ^{14}C-salicylic acid in the isolated perfused rat liver (mean weight 12.2 g, n = 2) at three different perfusate concentrations of salicylate at two flow rates.

Parameter	TRITIATED WATER			^{14}C-SALICYLIC ACID		
	Perfusate flow rate 20 ml min^{-1}					
Salicylate conc (mg l^{-1})	0	100	200	0	100	200
$f(t)_{max}$[a] ($\times 10^3$)	60	57	58	27	41	60
t_{max} (sec)	14.3	14.3	15.0	6.8	7.5	7.5
$AUC_{0-\infty}$ ratio[b]				0.99	1.02	1.04
MTT_H[c] (sec)	23.3	23.0	24.9	52.5	44.0	36.0
V_H (ml g^{-1})	0.64	0.63	0.69	1.45	1.20	0.99
	Perfusate flow rate 25 ml min^{-1}					
$f(t)_{max}$ ($\times 10^3$)	86	86	82	40	75	99
t_{max} (sec)	11.3	11.3	11.3	5.3	6.0	6.8
$AUC_{0-\infty}$ ratio[b]				0.97	1.02	0.99
MTT_H[c] (sec)	18.6	17.5	18.6	39.8	28.5	26.4
V_H (ml g^{-1})	0.64	0.60	0.64	1.37	0.98	0.90

[a] f(t): outflow fraction per second
[b] ratio of $AUC_{salicylate}/AUC_{tritiated\ water}$
[c] corrected for MTT_{NH}

of a solute on its hepatic distribution using statistical moment analysis and the axial dispersion model.

In the present investigation only total ^{14}C radioactivity was measured and assumed to be equivalent to ^{14}C-salicylic acid given that ^{14}C-salicylic acid is not eliminated when administered as a bolus into the single-pass IPRL. This assumption is based on results from preliminary HPLC analysis of unlabelled salicylic acid perfusate concentrations from Study II. These indicate that steady-state output was achieved within 15 min and recovery of unchanged salicylic acid was between 94% and 99%. This is in agreement with the very low hepatic extraction ratio (0.05) of salicylic acid in the rat (10,12).

In this study tritiated water was used as a reference to standardise the fractional outflow data for ^{14}C-salicylic acid under different experimental conditions (altered perfusate flow and salicylate concentrations). The inability of 100 and 200 mg l^{-1} salicylate in the perfusate to influence the frequency outflow profile, moment analysis statistics or dispersion model parameters of tritiated water following bolus injection suggests that this treatment does not disrupt the nature or viability of the isolated perfused rat liver preparation.

Ichikawa et al (14) have previously investigated the outflow profile of ^{14}C-salicylic acid following bolus input into the isolated perfusate rat liver, operated in the single-pass mode. ^{14}C-Salicylic acid displayed a multi-phasic outflow profile that was suggestive of non-instantaneous equilibra-

Table III. Mean (SD) parameter estimates for tritiated water and ^{14}C-salicylic acid frequency outflow data (n = 4) obtained at different flow rates fitted to a one-compartment (Equation 8) and two-compartment axial dispersion model (Equation 9), respectively.

	Tritiated water		^{14}C-salicylic acid			
Q[a]	D_N	V_H (ml)	D_N	k_{12} (sec^{-1})	k_{21} (sec^{-1})	Kp[b]
15	0.21 (0.04)	5.7 (0.6)	0.07 (0.05)	0.53 (0.04)	0.092 (0.01)	5.8 (1.1)
20	0.20 (0.04)	6.0 (0.3)	0.12 (0.05)	0.58 (0.09)	0.098 (0.02)	5.9 (1.9)
25	0.17 (0.04)	6.8 (0.9)	0.11 (0.07)	0.57 (0.08)	0.103 (0.04)	5.5 (1.9)
30	0.17 (0.04)	7.3 (1.2)	0.04 (0.01)	0.62 (0.12)	0.088 (0.02)	7.1 (2.8)
X (σ)[c]	0.19 (0.04)	6.4 (0.7)	0.08 (0.04)	0.56 (0.04)	0.095 (0.01)	6.1 (1.9)

[a] perfusate flow rate (ml min^{-1})
[b] apparent tissue-perfusate partition coefficient calculated as k_{12}/k_{21}
[c] group mean (X) and standard deviation (σ)

tion with the hepatic tissue. However, this was difficult to establish unambiguously due to the short duration of outflow collection time (only 20 sec) employed by these workers. Unlike the two-compartment model, in our experiments the one-compartment form of the axial dispersion model failed to adequately describe the temporal profile of ^{14}C-salicylate fractional outflow data obtained following a bolus injection (data not shown). This finding, supported by the observations from Ichikawa et al (14), suggests that the radial distribution of ^{14}C-salicylic acid in the liver, unlike tritiated water, is not instantaneous, but is limited by the permeability barrier at the level of the hepatic cell.

It has recently been demonstrated that the axial dispersion model is capable of accurately describing the kinetic behaviour of the highly cleared drugs diazepam (4) and diclofenac (3,5,8) in the IPRL under varied conditions of protein binding in the perfusate and alterations in perfusate flow rate during both impulse-response and steady-state experiments. The present work extends these findings by concluding that the two-compartment axial dispersion model can also account for the distribution kinetics of non-eliminated compounds under various alterations in the hepatic tissue binding.

The present finding, that parameters of the two-compartment dispersion model for ^{14}C-salicylic acid are independent of perfusate flow rate, is in contrast to data presented by Yano et al (17) for cefixime, using the same model. These workers found that both the D_N and V_H of cefixime in the isolated perfused rat liver tended to increase with increased flow rate while the Kp′ was reduced. This is in contrast to the observed lack of dependence of D_N on perfusate flow rate for compounds that display one-compartment characteristics (18). Furthermore, D_N was determined by the hepatic microvasculature, with the values of D_N the same for all compounds and equal to that of erythrocytes (6,7). One explanation for the apparent sensitivity of D_N of cefixime to changes in perfusate flow rate is a failure by the investigators to adequately correct for dispersion in the non-hepatic region of the experimental system. The importance of this correction when conducting kinetic studies in the liver has been emphasised (18,20).

Chou et al (21) recently demonstrated that the dispersion number (0.28 to 0.39) for a series of homologous barbiturates is independent of lipophilicity, lending further support to the proposal that dispersion in the liver is a characteristic of the organ's anatomical properties, such as the vascular architecture. The value of D_N determined for ^{14}C-salicylic acid in the present study using the two-

Table IV. Mean parameter estimates for tritiated water and ^{14}C-salicylic acid (n = 2) obtained from an individual fit using a one-compartment and simultaneous fit[a] of three data sets using a two-compartment axial dispersion model, respectively, at two flow rates.

Salicylate perfusate concentration (mg l^{-1})	Flow rate 20 ml min^{-1}				25 ml min^{-1}			
	TRITIATED WATER							
	D_N		V_H (ml)		D_N		V_H (ml)	
0	0.18		5.7		0.15		5.3	
100	0.18		5.9		0.15		5.2	
200	0.15		6.2		0.15		5.4	
	^{14}C-SALICYLIC ACID							
	D_N	k_{12} (sec^{-1})	k_{21} (sec^{-1})	Kp'[b]	D_N	k_{12} (sec^{-1})	k_{21} (sec^{-1})	Kp'[b]
0	0.08	0.45	0.071	6.3	0.09	0.45	0.080	5.7
100			0.096	4.7			0.134	3.4
200			0.134	3.3			0.235	1.9

[a] Using Reduced model I where $k_{12,A} = k_{12,B} = k_{12,C}$
[b] calculated as k_{12}/k_{21}

compartment dispersion model was consistently lower than has been observed for other solutes in recent work (3–7,21). The reason for this discrepancy has been sought. Simulations using dispersion model parameter estimates obtained in this study indicate that a failure to accurately characterise the throughput portion, especially the up-curve and $f(t)_{max}$, can lead to an underestimate of the true value of D_N. In practice, this problem arises when the collection interval of liver effluent is relatively large compared to the time taken for the throughput component to eluent. Simulating experimental collection conditions by discretizing simulated data (using a D_N a value in the range of 0.15 to 0.2, observed for tritiated water in these experiments and by others in the literature (2–5,21)) over 1.5 sec intervals it was possible to produce an outflow curve that was virtually identical to a profile simulated using a D_N of 0.08. Hence, the consistently lower value of D_N observed in the present study appears to have arisen due to the very rapid flux of ^{14}C-salicylic acid from the IPRL and limitations in the outflow fraction collection procedure.

An interesting observation in the present study is that the two-compartment dispersion model consistently underestimated the terminal portion of the ^{14}C-salicylic acid frequency outflow curve in the presence of 200 mg l^{-1} salicylate in the perfusate (Figure 2B). This observation was not a consequence of the choice of weighting scheme and occurred consistently for data obtained under these conditions. The reason for this finding is unclear.

Hepatocyte membrane permeability of salicylic acid based on k_{12} estimates

It is clear from the shape of frequency outflow profile and the suitability of a two-compartment dispersion model to describe the ^{14}C-salicylic acid data that a permeability barrier exists to retard the radial movement of this solute into hepatic tissue. It is possible to obtain an estimate of the permeability-area product (PS) for this compound from the first-order influx rate constant (k_{12}) using a rearrangement of Equation 10, assuming that the vascular volume of the liver V_B is 15% of liver weight (3–5) and given that fu (fraction unbound in the perfusate) is 1. The mean permeability-surface area product estimate for ^{14}C-salicylic acid was 4.6 ± 0.7 ml min^{-1} g^{-1} liver. Permeability estimates were independent of flow rate and of the concentration of salicylate in the perfusate. Interestingly, the value obtained from these *in situ* experiments is in close agreement with the literature estimate of the permeability-area product of salicylic acid determined in isolated hepatocyte uptake experiments conducted *in vitro* (6.4 ml min^{-1} g^{-1} liver (14)). These findings confirm that salicylate has a major permeability limitation in the liver and suggest that most likely it is at the hepatocyte membrane.

Fraction of salicylic acid bound in hepatic tissue

A volume of distribution of ^{14}C-salicylic acid approximately twice that of the total aqueous space of the liver implies that this compound has a significant affinity for hepatic tissue. Furthermore, the ability to reduce V_H by addition of salicylate to the perfusate would suggest that ^{14}C-salicylic acid undergoes considerable binding in the tissue. The liver-perfusate partition coefficient (Kp′) of approximately 6 with a tracer dose in the perfusate confirms this observation. The value of Kp′ obtained in the present experiments is in good agreement with *in vivo* unbound liver-plasma partition coefficients (Kp_u), equivalent to Kp′, presented in the literature. The estimate of Kp_u for liver, calculated based on data supplied by Hirate et al (12), was 6.4 following a dose of 10 mg kg^{-1} salicylic acid. This parameter was reduced to 1.7 when a higher salicylate dose was administered (173 mg kg^{-1}) (12). A similar value for liver ($Kp_u \approx 2$) was calculated from data presented by Yoshikawa et al (11), obtained in studies on the tissue distribution of salicylate in normal and pregnant rats. Yoshikawa et al (11) have suggested that both albumin and other proteins in the hepatic cytosol have a role in binding salicylic acid within the liver.

The absence of protein in the perfusate ($fu = 1$) provides an opportunity to investigate the extent of binding of ^{14}C-salicylic acid within the hepatic tissue. An estimate of the intracellular fraction unbound (fu_c) can be obtained from the two-compartment dispersion model parameter estimates by rearranging and combining Equations 10 and 11, to give,

$$fu_C = \frac{k_{21}\, V_C}{k_{12}\, V_B} \tag{13}$$

where V_c (defined as the aqueous cellular volume) is calculated as the difference between V_H of tritiated water (from the one-compartment dispersion model estimates in the same liver, Table IV) and V_B. This calculation assumes that salicylic acid distributes into the total water space within the liver and the influx and efflux permeability-surface area products are equal ($PS_{in} = PS_{out}$). The presence of salicylate in the perfusate results in a concentration-dependent decrease in the hepatic tissue binding of ^{14}C-salicylic acid (The values of fu_c were 0.37, 0.59 and 0.93 at 0, 100 and 200 mg l^{-1}, respectively).

CONCLUSIONS

The two-compartment dispersion model is capable of describing the impulse-response profile of salicylic acid in the isolated perfused rat liver under varying conditions of perfusate flow rate and hepatic tissue binding. The uptake of salicylic acid into the liver is governed by a major permeability barrier, most likely the hepatocyte membrane. Furthermore, an *in situ* estimate of PS can be obtained using the two-compartment dispersion model. These data are in good agreement with previous data for salicylic acid obtained *in vitro*. By performing impulse-response experiments with high concentrations of salicylate in the perfusate, the extent of uptake of salicylic acid in the rat liver has been shown to be saturable.

ACKNOWLEDGEMENT

One of us (AJM) was supported in part by a Merck (USA) Fellowship. The authors would like to thank Dr Yusuke Tanigawara for help with the implementation of the

MULTI(FILT) computer program and Chen-Hsi Chou for his valuable comments.

NOTE ADDED IN PROOF

Since this article was accepted, an article came out of *J. Pharmaceutical Sciences* (**83**:607–608, 1994) which supports our claim that salicylic acid is not metabolized during the first pass across the rat liver.

REFERENCES

1. G. R. Wilkinson. Clearance approaches in pharmacology. *Pharmacol. Rev.* **39**:1–45 (1987).
2. M. Rowland, D. Leitch, G. Fleming, B. Smith. Protein binding and hepatic clearance: discrimination between models of hepatic clearance with diazepam, a drug of high intrinsic clearance, in the isolated perfused rat liver preparation. *J. Pharmacokinet. Biopharm.* **12**:129–147 (1984).
3. A. M. Evans, Z. Hussein, M. Rowland. A two-compartment dispersion model describes the hepatic outflow profile of diclofenac in the presence of its binding protein. *J. Pharm. Pharmacol.* **43**:709–714 (1991).
4. J. M. Diaz-Garcia, A. M. Evans, M. Rowland. Application of the axial dispersion model of hepatic drug elimination to the kinetics of diazepam in the isolated perfused rat liver. *J. Pharmacokinet. Biopharm.* **20**:171–193 (1992).
5. A. M. Evans, Z. Hussein, M. Rowland. Influence of albumin on the distribution and elimination of diclofenac in the isolated perfused rat liver: Analysis by impulse-response technique and the dispersion model. *J. Pharm. Sci.* **82**:421–428 (1993).
6. M. Rowland, A. M. Evans. Physiologic models of hepatic elimination. *New Trends in Pharmacokinetics*. Eds A. Rescigno, A. K. Thakur. Plenum Press, New York, 1991.
7. M. S. Roberts, M. Rowland. A dispersion model of hepatic elimination. 1. Formulation of the model and bolus considerations. *J. Pharmacokinet. Biopharm.* **14**:227–260 (1986).
8. Z. Hussein, A. M. Evans, M. Rowland. Physiologic models of hepatic clearance: Influence of altered protein binding on the elimination of diclofenac in the isolated perfused rat liver. *J. Pharm. Sci.* **82**:880–885 (1993).
9. Y. Yano, K. Yamaoka, Y. Aoyama, H. Tanaka. Two-compartment dispersion model for analysis of organ perfusion system of drugs by fast inverse laplace transform (FILT). *J. Pharmacokinet. Biopharm.* **17**:179–202 (1989).
10. E. Nelson, M. Hanano, G. Levy. Comparative pharmacokinetics of salicylate elimination in man and rats. *J. Pharmacol. Exp. Ther.* **153**:159–166, 1966
11. T. Yoshikawa, Y. Sugiyama, Y. Sawada, T. Iga, M. Hanano. Effect of pregnancy on tissue distribution of salicylate in rats. *Drug Metab. Dispos.* **12**:500–505 (1984).
12. J. Hirate, Y. Kato, I. Horikoshi, S. Nagase, C. T. Ueda. Further observations on the disposition characteristics of salicylic acid in analbuminemic rats. *Biopharm. Drug Dispos.* **10**:299–309 (1989).
13. S. H. Dromgoole, D. E. Furst. Salicylates. In: *Applied Pharmacokinetics* Eds: W. E. Evans, J. J. Schentag, W. J. Jusko. Applied Therapeutic Inc., Spokane, WA, 1986.
14. M. Ichikawa, S. C. Tsao, T-H. Lin, S. Miyauchi, Y. Sawada, T. Iga, M. Hanano, Y. Sugiyama. Albumin-mediated transport phenomenon observed for ligands with high permeability: Effect of unstirred water layer in the disse's space of the rat liver. *Hepatol.* **16**:38–49 (1992).
15. B. E. Cham, D. Johns, F. Bochner, D. M. Imhoff, M. Rowland. Simultaneous liquid-chromatographic separation of salicylic acid, salicyluric acid and gentisic acid in plasma. *Clin. Chem.* **25**:1420–1425, (1979).
16. H. G. Boxenbaum, S. Riegelman, R. M. Elashoff. Statistical estimations in pharmacokinetics. *J. Pharmacokinet. Biopharm.* **2**:123–148 (1974).
17. Y. Yano, K. Yamaoka, H. Yasui, T. Nakagawa. Effect of perfusion rate on the local disposition of cefixime in liver perfusion system based on two-compartment dispersion model. *Drug Metab. Dispos.* **19**:1022–1027 (1991).
18. C. A. Goresky. Kinetic interpretation of hepatic multiple-indicator dilution studies. *Am. J. Physiol.* **245**:G1–G12 (1983).
19. M. S. Roberts, S. Fraser, A. Wagner, L. McLeod. Residence time distribution of solutes in the perfused rat liver using a dispersion model of hepatic elimination: 1. Effect of changes in perfusate flow rate and albumin concentration on sucrose and taurocholate. *J. Pharmacokinet. Biopharm.* **18**:209–234 (1990).
20. C. A. Goresky, M. Silverman. Effect of correction of catheter distortion on calculated liver sinusoidal volumes. *Am J. Physiol.* **207**:883 (1964).
21. C-H. Chou, A. M. Evans, G. Fornasini, M. Rowland. Relationship between lipophilicity and hepatic dispersion and distribution for a homologous series of barbiturates in the isolated perfused *in situ* rat liver. *Drug Metab. Dispos.* **21**:933–938 (1993).