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Evaluation of Protein Binding Effect on Local Disposition of Oxacillin in Rat Liver by a Two-compartment Dispersion Model

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Abstract—The effect of protein binding upon the hepatic uptake of oxacillin was evaluated in the rat isolated perfused liver, based on the two-compartment dispersion model by means of the fast inverse Laplace transform (FILT). The perfusion experiment was carried out using the perfusates without and with bovine serum albumin (BSA, 40 g L^{-1}). Oxacillin was injected as a pulse through the portal vein, and the outflow concentration-time course of oxacillin was fitted to the dispersion model using the non-linear least squares program MULTI(FILT). The partition ratio (k′), which is the measure of the extent of the reversible distribution into the hepatic tissue, was $0{\cdot}163 \pm 0{\cdot}041$ (s.d.) in the presence of BSA, and $0{\cdot}095 \pm 0{\cdot}018$ in the absence of BSA, which suggests interaction of the albumin-bound drug with the hepatic tissue. The elimination rate constant (k_e) from the perfusate in the absence of BSA was $8{\cdot}0 \pm 0{\cdot}55$ min^{-1} and that in the presence of BSA was $3{\cdot}3 \pm 1{\cdot}4$ min^{-1} while the unbound fraction of the drug in the presence of 40 g L^{-1} BSA was 0·282. The hepatic elimination rate of oxacillin was not proportional to the unbound concentration of drug suggesting hepatic uptake of the bound fraction.

Protein binding is a physiological phenomenon that affects drug disposition and it is often assumed that only the unbound drug can be related to elimination and the pharmacological effect (Coffey et al 1971; McNamara et al 1979; Øie & Tozer 1979). Physiological one-organ models based on the clearance concept have been adopted to explain the role of protein binding in tissue elimination (Wilkinson & Shand 1975; Pang & Rowland 1977; Rowland et al 1984). Those models (i.e. the well-stirred model and the parallel tube model), are usually adopted with assumption of steady-state conditions (Forker & Luxon 1981, 1983a, b). Forker et al (1982) and Weisiger et al (1981) reported that the hepatic uptake rate is not proportional to the fraction unbound and that the bound fraction of a drug is also extracted by the liver.

To examine precisely the effect of protein binding on the drug transfer process, an analysis of the outflow profile with non-steady-state condition (i.e. single-pass bolus) is useful. In this report, we performed a single-pass bolus perfusion study and applied the two-compartment dispersion model to the analysis of the outflow pattern to evaluate the effect of protein binding on the uptake and elimination of oxacillin in the rat perfused liver.

We have recently introduced two dispersion models, i.e. one-compartment and two-compartment models (Yano et al 1989a), which were originally developed in chromatography (Yamaoka & Nakagawa 1976). The mass balance equations in these dispersion models are described by second-order partial differential equations, and are often solved by means of the Laplace transform. As the analytical inversion of the Laplace-transformed equations into the time domain is often difficult or almost impossible, curve fitting of the outflow data of the perfusion experiments was carried out based on the Laplace-transformed equations (i.e. image equations) by means of the non-linear least-squares program MULTI-(FILT) which we have recently developed (Yano et al 1989b). From the pharmacokinetic parameters obtained by MULTI-(FILT), the moment characteristics, recovery ratio F_H, mean hepatic transit time $\bar{t}_H$, and relative dispersion $\sigma^2/\bar{t}_H^2$ were calculated. The dispersion number (D_N) and the efficiency number (R_N) (Roberts & Rowland 1985a, b, 1986a) were also calculated.

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Theory

All the disposition processes in the liver are assumed to be linear. In the one-compartment dispersion model, the instantaneous equilibrium of a drug is assumed between the perfusate and liver tissue, while in the two-compartment dispersion model, the partition of the drug at a finite velocity is assumed between the perfusate and liver tissue. The differential equation for the one-compartment dispersion model is equivalent to that for the organ perfusion model proposed by Roberts & Rowland (1985a, 1986a, b), though the initial and boundary conditions are different. The details of the one- and two-compartment dispersion models have been described previously (Yano et al 1989a).

Fig. 1 gives the schematic representation of the two-compartment dispersion model where the elimination is assumed to take place from the central compartment. Equation 1 is the Laplace-transformed solution of the outflow profile after the impulse input of a drug:

$$\tilde{C}_1(s) = \frac{M}{Q}\exp\left[\left\{\frac{Q}{2D_c} - \sqrt{\left(\frac{Q}{2D_c}\right)^2 + \frac{1}{D_c}\left\{s + k_{12} + k_e - \frac{k_{12}\cdot k_{21}}{s + k_{21}}\right\}}\right\}V_B\right] \quad (1)$$

Where M is the amount of dose, Q is the flow rate of the perfusate, D_c is the corrected dispersion coefficient, and k_{12}

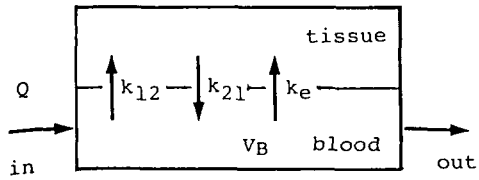

FIG. 1. Schematic representation of the two-compartment dispersion model. Q: perfusate flow rate; k_{12}, k_{21}: forward and backward partition rate constants, respectively, k_e: elimination rate constant from central compartment; V_B: volume of the blood space.

and k_{21} are the forward and backward transfer rate constants, respectively. The partition ratio (k') is defined by k_{12}/k_{21}. The coefficient k_e is defined as first-order elimination (or irreversible transfer) rate constant from the central compartment (perfusate) mainly into the hepatic tissues. The parameter V_B is the blood volume which corresponds to the sum of the volume of the sinusoid and the space of Disse, as drugs (including albumin) can rapidly distribute into the space of Disse. The detailed discussions concerning the boundary conditions and the site of elimination were reported previously (Yano et al 1989a). The moments of the outflow curve, i.e. the recovery ratio F_H, mean transit time ($\bar{t}_H$), and the relative variance ($\sigma^2/\bar{t}_H^2$) are given by equations (2)–(4).

$$F_H = \frac{\lim_{s\to 0} \tilde{C}_1(s)}{M/Q} = \exp\left[\left\{\frac{Q}{2D_c} - \sqrt{\left(\frac{Q}{2D_c}\right)^2 + \frac{k_e}{D_c}}\right\}V_B\right] \quad (2)$$

$$\bar{t}_H = \lim_{s\to 0}\frac{-d}{ds}\ln\tilde{C}_1(s) = \frac{V_B}{Q}(1+k')\left\{1+\frac{4D_c\cdot k_e}{Q^2}\right\}^{-\frac{1}{2}} \quad (3)$$

$$\sigma^2/\bar{t}_H^2 = \lim_{s\to 0}\frac{d^2}{ds^2}\ln\tilde{C}_1(s)/\bar{t}_H^2 = \left[V_B\left\{\left(\frac{Q}{2D_c}\right)^2+\frac{k_e}{D_c}\right\}^{\frac{1}{2}}\right]^{-1} + 2\frac{k_{12}}{k_{21}^2}\cdot\left\{\left(\frac{Q}{2D_c}\right)^2+\frac{k_e}{D_c}\right\}^{\frac{1}{2}}\cdot\left\{\frac{V_B}{2D_c}(1+k')^2\right\}^{-1} \quad (4)$$

For the one-compartment dispersion model where the equilibrium partition of the drug is assumed, the Laplace-transformed equation is given by;

$$\tilde{C}(s) = \frac{M}{Q}\exp\left[\left\{\frac{Q}{2D_c} - \sqrt{\left(\frac{Q}{2D_c}+\frac{(1+k')s+k_e}{D_c}\right)}\right\}V_B\right] \quad (5)$$

where k' is the partition ratio. The parameters in the one- and two-compartment dispersion models are related to the dispersion number D_N and the efficiency number R_N as follows.

$$D_N = D_c/(Q\cdot V_B) \quad (6)$$
$$R_N = k_e\cdot V_B/Q \quad (7)$$

Methods and Materials

Numerical procedure

The non-linear regression program MULTI(FILT) was developed on the mainframe computer M-382 of Kyoto University Data Processing Center. This program is written in FORTRAN77 and is available for many personal computers and mainframe computers. MULTI(FILT) can numerically inverse the Laplace-transform equations defined, according to FILT algorithm (Hosono 1981) and can estimate the pharmacokinetic parameters by non-linear curve fitting to the experimental data points.

Animal experiments

Single-pass perfusion experiments using a rat isolated liver were performed according to the Mortimore perfusion method (Mortimore & Tietze 1959; Mortimore et al 1959). Male Wistar rats, 210–240 g, were anaesthetized with pentobarbitone (Nembutal, Abbot Lab., USA) and the bile duct was cannulated with a polyethylene tube (0·67 mm o.d.). Perfusates of Krebs-Ringer-bicarbonate buffer containing 10 mM glucose, without and with bovine serum albumin (BSA, 40 g L^{-1}) were used. During the experiment, the buffer (pH 7·4) was saturated with 95% O_2–5% CO_2 and kept at 37 °C. The portal vein was rapidly catheterized with a polyethylene cannula (1·67 mm o.d.) and the perfusate containing no BSA was delivered through the cannula into the liver by a roller pump (RP-N3, Furue Sci. Co. Ltd. Japan). After a stabilization period of 10 min, 0·245 mL of oxacillin solution (1·25 mg mL^{-1}) was introduced into the portal vein using a six-position rotary valve injector and the outflow samples were collected at approximately 1 s intervals from the cannula inserted into the inferior vena cava. The exact sampling time was calculated from the eluent volume of each sample based on the assumption that the flow rate is constant. After the end of the first run, the perfusate was changed to the buffer containing 40 g L^{-1} BSA and the liver was stabilized for more than 10 min. The second run was then started with 0·24 mL of oxacillin solution (1·25 mg mL^{-1}) in the buffer containing 40 g L^{-1} BSA. The flow rate of the perfusate was maintained at 14·9 mL min^{-1} (±0·15 s.d.). The flow recovery was 99·7% (±1·46 s.d.). The viability of the liver was monitored by bile flow throughout the experiments. The wet liver weight was 8·7 g (±0·92 s.d.). The outflow concentration of the drug was measured by the high performance liquid chromatographic (HPLC) method described below. The void time through the inlet and outlet catheter was subtracted from the outflow profile. The variance of the catheter transit time was less than 0·09 s^2 which is about 1·7% or less compared with the variance of the liver perfusion data. Thus, the broadening of the injected sample in the injector loop and the catheter was negligible.

Chemicals

Oxacillin (Staphcillin V) was obtained from Banyu Pharm. Co. Ltd (Tokyo, Japan). Bovine serum albumin (Fraction V) was purchased from Nacalai Tesque (Kyoto, Japan). All other reagents for the Krebs-Ringer-bicarbonate buffer and for the mobile phase in HPLC were of analytical grade.

Analytical procedure

A high performance liquid chromatograph (LC-5A, Shi-

madzu Co., Kyoto, Japan) equipped with a UV-detector SPD-2A Shimadzu) and an integrated data analyzer (Chromatopac C-R3A, Shimadzu) was used with a stationary phase of Chemosorb 5-ODS-H (Chemco. Co., Osaka, Japan) for the determination of oxacillin concentration in the outflow samples. A guard column (50 × 4·6 mm i.d.) was packed with LiChrosorb RP-2 (E. Merck). The mobile phase, acetate buffer (pH 5·2)—MeOH (1:1 v/v), was delivered at 1·0 mL min^{-1}. The wavelength was set at 220 nm, and the column temperature was kept at 40°C.

Acetonitrile (0·3 mL) was added to 0·2 mL of the outflow sample to precipitate protein, and the mixture was centrifuged; 15 μL of the supernatant was injected into the HPLC.

Protein binding

The binding of oxacillin was determined in the perfusate containing various concentrations of BSA at 37°C by the ultrafiltration method. A disposable ultrafiltration kit, Molcut II (UFP1 LGC, Millipore Co. USA), was used to determine the free drug concentration. BSA was dissolved in Krebs-Ringer bicarbonate buffer (pH 7·4), to give concentrations of BSA of 0, 5, 10, 20 and 40 g L^{-1}. A portion of the filtrate containing free oxacillin was obtained by displacing 0·4 mL of the oxacillin-BSA mixture with 4 mL of air. Oxacillin concentrations were varied from 2 to 500 μg mL^{-1}. All filtration procedures were performed at 37°C. The filtrate was directly injected into the HPLC system, and the unbound oxacillin concentration was measured. Adsorption of the drug onto the surface of the filter membrane was negligible. The total (unbound plus bound) drug concentration was measured by the same method using acetonitrile for the precipitation of BSA as described in the analytical procedure.

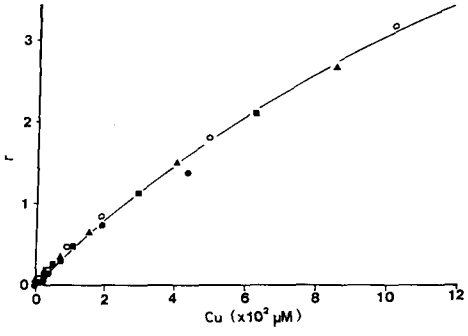

FIG. 2. Langmuir plot of oxacillin binding to BSA. $r = C_b/[Pt]$, where [Pt] is the concentration of BSA in the perfusate, C_u and C_b are unbound concentration of oxacillin, respectively. BSA concentrations are 5 (○), 10 (▲), 20 (■), 40 (●) g L^{-1}. The line is the result of the non-linear curve fitting by MULTI.

Results

Protein binding

The in-vitro data were fitted to equation 8 using a non-linear regression program MULTI (Yamaoka et al 1981);

$$C_b/[Pt] = n \cdot C_u/(K_d + C_u) \quad (8)$$

where [Pt] is the concentration of BSA (μM) in the perfusate, C_b and C_u are the bound and unbound concentration (μM) of oxacillin. Fig. 2 is the Langmuir plot and the curve by the best fit of equation 8. The dissociation constant (k_d) and the number of binding sites (n) were estimated to be 2·53 and 10·7 mM, respectively. The unbound fraction in the presence of 40 g L^{-1} BSA was thus estimated as 0·282 according to the relationship, $f_u = K_d/(K_d + n \cdot [Pt])$.

Result of curve fitting

Fig. 3 shows the typical outflow pattern and the result of curve fitting by MULTI(FILT) to the one-compartment and the two-compartment dispersion models. In the terminal phase the one-compartment dispersion model is not a good fit for the data. The model estimation by the Akaike's information criterion (AIC) (Yamaoka et al 1978) supports the two-compartment dispersion model to describe the outflow profile of oxacillin in the presence and in the absence of albumin. The parameters obtained by the curve fitting to the two-compartment dispersion model are listed in Table 1. The moment characteristics were calculated from these parameters using equations 2–4. These moment values based on the two-compartment dispersion model were in good agreement with those calculated by numerical integration according to the trapezoidal rule.

The corrected dispersion coefficient D_c and the blood volume V_B were not significantly different by ANOVA ($P > 0.05$) between experiments with and without albumin. Consequently, the dispersion number D_N was not significantly different. These results suggest that the D_c and V_B values depend largely on the structure of the sinusoid and the

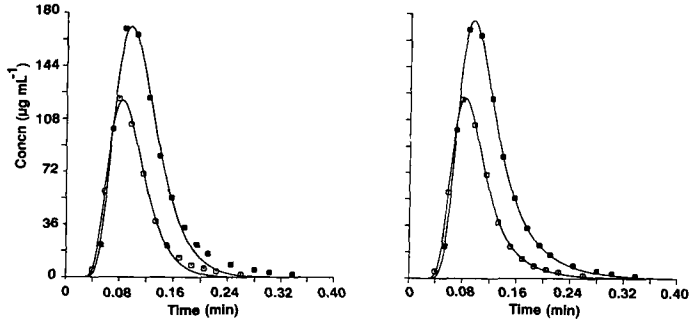

FIG. 3. Typical oxacillin concentration vs. time data in the presence of 40 g L^{-1} BSA (●) and in the absence of BSA (○), and the lines of least squares fitting by MULTI(FILT) to a one-compartment dispersion model (left panel) and a two-compartment dispersion model (right panel).

Table 1. Curve fitting to two-compartment dispersion model using MULTI(FILT).

	Without albumin (n=6)		With albumin (n=6)	
	mean	s.d.	mean	s.d.
D_c (mL^2/min^{-1})	1·02	0·269	1·13	0·196
V_B (mL)	1·62	0·121	1·66	0·117
k_{12} (min^{-1})	2·46	0·665	4·39	1·57
k_{21} (min^{-1})	25·5	2·79	26·4	4·06
k' ($=k_{12}/k_{21}$)	0·095	0·018	0·163	0·041
k_e (min^{-1})	8·03	1·44	3·28	0·55
D_N	0·042	0·009	0·045	0·005
R_N	0·867	0·152	0·368	0·075
F_H (%)	43·7	6·55	69·9	5·09
$\bar{t}_H$ (s)	6·7	0·41	7·5	0·46
$\sigma^2/\bar{t}_H^2$	0·140	0·015	0·172	0·019
% V_B to liver*	18·9	2·33	19·4	2·73

* Estimated from V_B and wet liver weight.

Notation:

$\bar{C}(s)$	Laplace transform of the outflow profile
C_b, C_f	bound and unbound concentration of drug
D_c	corrected dispersion coefficient
D_N	dispersion number
F_H	recovery ratio
k'	partition ratio (distribution ratio)
k_{12}, k_{21}	forward and backward partition rate constants
k_e	elimination (or irreversible transfer) rate constant
M	amount of drug injected
[Pt]	concentration of protein (BSA)
Q	flow rate of perfusate
R_N	efficiency number
s	Laplace variable
$\bar{t}_H$	mean transit time
V_B	blood volume
σ^2	variance of transit time
$\sigma^2/\bar{t}_H^2$	relative dispersion to transit time

Table 2. Comparison of the predicted and experimental values of k_e and F_H.

	Predicted		Experimental	
	mean	s.d.	mean	s.d.
k_e (min^{-1})	2·27	0·41	3·28	0·55
R_N[a]	0·245	0·046	0·368	0·075
F_H (%)[b]	78·6	3·35	69·9	5·09

The predicted k_e value is obtained by multiplying the experimentally obtained k_e of the albumin-free system (Table 1) by the fraction unbound ($f_u=0·282$).

[a] Calculated using eqn (7) and predicted k_e.

[b] Calculated using eqn (2) and predicted k_e.

space of Disse and are independent of the perfusion medium. The estimated V_B, which corresponds to the sum of the volume of sinusoid plus the space of Disse, was 1·64 mL (±0·12 s.d.), i.e. 19·1% (±2·55 s.d.) per g wet liver, and coincides with the estimate by Goresky (1963).

The backward partition rate constant, k_{21}, was not significantly different between experiments with and without albumin. The forward partition rate constant, k_{12}, in the presence of albumin was about 1·8-fold greater than that in its absence. Thus, the partition $k'(=k_{12}/k_{21})$ in the albumin system was greater than that in the albumin-free system, despite the small free fraction ($f_u=0·282$). These results suggest that not only the free fraction of oxacillin but a considerable part of the protein-bound drug reversibly partitioned into the peripheral compartment. This partition process described by k_{12} (or k') includes the interaction of the albumin-drug complex with the cell membrane and the facilitated transfer of the bound fraction into the hepatocytes.

Table 2 shows the comparison of the experimentally obtained parameters from albumin experiments and the predicted values from albumin-free experiments and f_u values. The prediction was based on the assumption that only the unbound fraction can reversibly or irreversibly transfer into the hepatic tissue. The experimentally obtained k_e in the albumin system is about 1·4-fold greater than the predicted k_e. The efficiency number R_N and the recovery ratio (F_H) are also significantly different between the observed and the predicted values. If the elimination process occurs only within the hepatocytes, this result would contradict the assumption that only the free fraction passes through into the hepatic tissue cells. It is therefore suggested that the bound fraction of drug may also be subject to hepatic elimination.

Discussion

To explain the hepatic uptake of a drug, two well-defined models, i.e. the well-stirred model and the parallel-tube model, have been proposed. These models are the extremes of the dispersion model (Roberts & Rowland 1985a; Yano et al 1989a) and are not appropriate to describe the precise shape of the outflow pattern after the bolus input of a drug. In the adaptation of these simplified models, the steady-state experimental condition has usually been assumed, and the effect of protein binding on hepatic drug extraction has been examined with the continuous infusion or recirculation experimental system. Most reports dealing with so-called 'protein-mediated uptake' are based on these steady-state experimental conditions (Forker & Luxon 1981, 1983a; Weisiger et al 1981; Forker et al 1982), and comparison and discrimination of these models have been reported (Colburn 1982; Morgan & Raymond 1982; Forker & Luxon 1983b; Jones et al 1984; Smallwood et al 1988). As suggested by Tsao et al (1986), the parameters estimated under the steady-state conditions are hybrid parameters and are not suitable to model the forward transport from the perfusate into the liver tissue and the backward transport from the liver tissue into the perfusate. Thus, they performed single-pass bolus studies using the multiple indicator dilution method (Tsao et al 1986, 1988). We also carried out a single-pass bolus study and separately estimated the reversible partition ratio (k') and irreversible transfer rate constant (k_e) by non-linear curve fitting. The $\sigma^2/\bar{t}_H^2$ reflects both the dispersion process and the non-equilibrium distribution process in the two-compartment dispersion model. The dispersion and the non-equilibrium processes can be separately evaluated based on the two-compartment dispersion model.

By the analysis of the two-compartment dispersion model with a single-pass bolus study, it is concluded that the albumin-bound drug interacts reversibly with the hepatic tissue and is eliminated from the perfusate. In equation 3, the mean transit time is mainly affected by k' in the dispersion model (Yano et al 1989a). The greater mean hepatic transit

time ($\bar{t}_H$) in the albumin system reflects an interaction of the bound fraction with the hepatic tissue (i.e. the large value of k′ in the presence of albumin). The distribution volume in the liver, which is approximated by $V_B(1+k')$, was 1·77 and 1·93 mL in the absence and in the presence of BSA, respectively. Both the removal of the unbound drug by the hepatic tissue and the presumed interaction of the albumin with the hepatic tissue would accelerate the dissociation of the albumin-drug complex and this would lead to the hepatic uptake of the bound-fraction.

It has been reported that oxacillin is rapidly and extensively biotransformed to active and inactive metabolites (Murai et al 1981). Barza & Weinstein (1976) reported that about half of the orally administered oxacillin is metabolized in man, which suggests a large hepatic extraction despite the small free fraction ($f_u = 0{\cdot}1$ in serum). The result of the present perfusion experiment is in accordance with the in-vivo study.

As the analytic inversion of the Laplace-transformed model equation (eqn 1) is difficult or almost impossible, the FILT method is useful for the curve fitting of the outflow data to the dispersion model. One of the advantages of the FILT method is that the relationship of the pharmacokinetic model parameters and the moment characteristics can be simply correlated.

References

Barza, M., Weinstein, L. (1976) Pharmacokinetics of the penicillins in man. Clin. Pharmacokinet. 1: 297–308

Coffey, J. J., Bullock, F. J., Schoenemann, P. T. (1971) Numerical solution of nonlinear pharmacokinetic equations: effect of plasma protein binding on drug distribution and elimination. J. Pharm. Sci. 60: 1623–1628

Colburn, W.A. (1982) Albumin does not mediate the removal of taurocholate by the rat liver. Ibid. 71: 373–374

Forker, E. L., Luxon, B. A. (1981) Albumin helps mediate removal of taurocholate by rat liver. J. Clin. Invest. 67: 1517–1522

Forker, E. L., Luxon, B. A. (1983a) Albumin mediated transport of rose bengal by perfused rat liver. Ibid. 72: 1764–1771

Forker, E. L., Luxon, B. A. (1983b) Albumin binding and hepatic uptake: the importance of model selection. J. Pharm. Sci. 72: 1232–1233

Forker, E.L., Luxon, B. A., Snell, M., Hurmantine, W. O. (1982) Effect of albumin binding on the hepatic transport of rose bengal: surface-mediated dissociation of limited capacity. J. Pharmacol. Exp. Ther. 223: 342–347

Goresky, C. A. (1963) A linear method for determining liver sinusoidal and extravascular volumes. Am. J. Physiol. 204: 626–640

Hosono, T. (1981) Numerical inversion of Laplace transform and some applications to wave optics. Radio Sci. 16: 1015–1019

Jones, D. B., Morgan, D. J., Mihaly, G. W., Webster, L. K., Smallwood, R. A. (1984) Discrimination between the venous equilibrium and sinusoidal models of hepatic elimination in the isolated perfused rat liver by perturbation of propranolol protein binding. J. Pharmacol. Exp. Ther. 229: 522–526

McNamara, P. J., Levy, G., Gibaldi, M. (1979) Effect of plasma protein and tissue binding on the time course of drug concentration in plasma. J. Pharmacokinet. Biopharm. 7: 195–206

Morgan, D. J., Raymond, K. (1982) Use of unbound drug concentration in blood to discriminate between two models of hepatic drug elimination. J. Pharm. Sci. 71: 600–602

Mortimore, G. E., Tietze, F. (1959) Studies on the mechanism of capture and degradation of insulin-I^{131} by the cyclically perfused rat liver. Ann. N. Y. Acad. Sci. 82: 329–337

Mortimore, G. E., Tietze, F., Stellen, D. (1959) Metabolism of insulin-I^{131}. Studies in isolated perfused rat liver and hind-limb preparations. Diabetes 8: 307–314

Murai, Y., Nakagawa, T., Yamaoka, K., Uno, T. (1981) High performance liquid chromatographic analysis and pharmacokinetic investigation of oxacillin and its metabolites in man. Chem. Pharm. Bull 29: 3290–3298

Øie, S., Tozer, T. N. (1979) Effect of altered plasma protein binding on apparent volume of distribution. J. Pharm. Sci. 68: 1203–1205

Pang, K. S., Rowland, M. (1977) Hepatic clearance of drugs. I. Theoretical consideration of plasma, and blood cell binding, and the hepatocellular enzymatic activity on hepatic drug clearance. J. Pharmacokinet. Biopharm. 5: 625–653

Roberts, M.S., Rowland, M. (1985a) Hepatic elimination: dispersion model. J. Pharm. Sci. 74: 585–587

Roberts, M. S., Rowland, M. (1985b) Correlation between in-vitro microsomal enzyme activity and whole organ hepatic elimination kinetics: analysis with a dispersion model. J. Pharm. Pharmacol. 38: 177–181

Roberts, M. S., Rowland, M. (1986a) A dispersion model of hepatic elimination: 1. Formulation of the model and bolus consideration. J. Pharmacokinet. Biopharm. 14: 227–260

Roberts, M. S., Rowland, M. (1986b) A dispersion model of hepatic elimination: 2. Steady-state considerations—influence of hepatic blood flow, binding within blood, and hepatocellular enzyme activity. Ibid. 14: 261–288

Rowland, M., Leitch, D., Fleming, G., Smith, B. (1984) 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. Ibid. 12: 129–147

Smallwood, R. H., Morgan, D. J., Mihaly, G. W., Jones, D. B., Smallwood, R. A (1988) Effect of plasma protein binding on elimination of taurocholate by isolated perfused rat liver: comparison of venous equilibrium, undistributed and distributed sinusoidal, and dispersion models. Ibid. 16: 377–396

Tsao, S. C., Sugiyama, Y., Sawada, Y., Nagase, S., Iga, T., Hanano, M. (1986) Effect of albumin on hepatic uptake of warfarin in normal and analbuminemic mutant rats: analysis by multiple indicator dilution method. Ibid. 14: 51–64

Tsao, S. C., Sugiyama, Y., Sawada, Y., Iga, T., Hanano, M. (1988) Kinetic analysis of albumin-mediated uptake of warfarin by perfused rat liver. Ibid. 16: 165–181

Weisiger, R., Gollan, J., Ockner, R. (1981) Receptor for albumin on the liver cell surface may mediate uptake of fatty acids and other albumin-bound substances. Science 211: 1048–1051

Wilkinson, G. R., Shand, D. B. (1975) A physiological approach to hepatic drug clearance. Clin. Pharmacol. 18: 377–390

Yamaoka, K., Nakagawa, T. (1976) Moment analysis for reaction chromatography. J. Chromatogr. 117: 1–10

Yamaoka, K., Nakagawa, T., Uno, T. (1978) Application of Akaike's Information Criterion (AIC) in the evaluation of linear pharmacokinetic equations. J. Pharmacokinet. Biopharm. 6: 165–175

Yamaoka, K., Tanigawara, Y., Nakagawa, T., Uno, T. (1981) A Pharmacokinetic analysis program (MULTI) for microcomputer. J. Pharmacobio-Dyn. 4: 879–885

Yano, Y., Yamaoka, K., Aoyama, Y., Tanaka, H. (1989a) Two-compartment dispersion model for analysis or organ perfusion system of drug by fast inverse Laplace transform (FILT). J. Pharmacokinet. Biopharm. 17: 179–202

Yano, Y., Yamaoka, K., Tanaka, H. (1989b) A non-linear least squares program, MULTI(FILT), based on fast inverse Laplace transform for microcomputers. Chem. Pharm. Bull. 37: 1535–1538