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Interconnected-Tubes Model of Hepatic Elimination: Steady-state Considerations

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In the interconnected-tubes model of hepatic transport and elimination, intermixing between sinusoids was modelled by the continuous interchange of solutes between a set of parallel tubes. In the case of strongly interconnected tubes and for bolus input of solute, a zeroth-order approximation led to the governing equation of the dispersion model. The dispersion number was expressed for the first time in terms of its main physiological determinants: heterogeneity of flow and density of interconnections. The interconnected-tubes model is now applied to steady-state hepatic extraction. In the limit of strong interconnections, the expression for output concentrations is predicted to be similar in form to those predicted by the distributed model for a narrow distribution of elimination rates over sinusoids, and by the dispersion model in the limit of a small dispersion number D_N. More generally, the equations for the predicted output concentrations can be expressed in terms of a dimensionless 'heterogeneity number' H_N, which characterizes the combined effects of variations in enzyme distribution and flow rates between different sinusoids, together with the effects of interconnections between sinusoids. A comparative analysis of the equations for the dispersion and heterogeneity numbers shows that the value of H_N can be less than, greater than or equal to the value of D_N for a correlation between distributions of velocities and elimination rates over sinusoids, anticorrelation between them, and when all sinusoids have the same elimination rate, respectively. Simple model systems are used to illustrate the determinants of H_N and D_N.

1. Introduction

The prediction of hepatic extraction in terms of changes in hepatic blood flow, solute binding in the blood and hepatic enzyme activity is of clinical importance. The well-stirred model has been widely used as illustrated in the recent work on the prediction of hepatic extraction under varying conditions of cardiac output (Schoemaker *et al.*, 1998); the dispersion model has also been widely applied, especially in the prediction of human hepatic extraction of solutes from their *in vitro* metabolic clearances in human hepatocytes or microsomes (Iwatsubo *et al.*, 1997); and the distributed model has been used to predict various aspects of steady-state extraction (Bass, 1980; Bass & Keiding, 1988).

Whilst the well-stirred model represents the liver as one completely mixed compartment or tank, the dispersion model represents it as

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a "chemical reactor", and aims to take account of (1) the variations in the flow velocity and in the lengths of different sinusoids, (2) mixing of blood at the branch points of sinusoids and at the interconnections between sinusoids, and (3) in principle, molecular diffusion, in terms of a "dispersion number" D_N, average flow-velocity v_{av}, and average elimination rate k_{av} appearing in a partial differential equation of convection–diffusion–reaction form. A limitation of the dispersion model has been the limited understanding and mathematical description of its defining stochastic parameter, the dispersion number D_N. To date, almost all studies exploring the determinants and magnitude of D_N have been experimental, measuring outflow concentrations after injection into the liver. Such studies have included bolus inputs of vascular references (Roberts & Rowland, 1986a), steady-state extraction (Roberts & Rowland, 1986b), metabolite formation (Roberts & Rowland, 1986c) and microsomal enzyme clearances (Roberts & Rowland, 1986d). Bass *et al.* (1987) and Roberts *et al.* (1988) recognized that the dispersion number was determined by vascular dispersion and enzyme distribution along the liver sinusoids. Vascular dispersion, which can be determined by the outflow concentration–time profile of a vascular reference, has been shown to be relatively independent of hepatic flow rate and blood composition (Roberts *et al.*, 1990a), coadministration of vasoactive substances (Roberts *et al.*, 1990b), age and species differences (Roberts *et al.*, 1999), hepatic regeneration after partial hepatectomy (Weiss *et al.*, 1998) and ischaemia reperfusion (unpublished data). Whilst enzyme heterogeneity (Bass *et al.*, 1987; Roberts *et al.*, 1988), axial diffusion (Rivory *et al.*, 1992) and radial tissue diffusion (Luxon & Weisiger, 1993; Weiss & Roberts, 1996) affect interpretation of the dispersion number, most recent experimental studies have found that the dispersion number for extracted solutes, D_N^E, is similar to that for vascular dispersion, D_N^V, for bolus input experiments (Chou *et al.*, 1993; Hussein *et al.*, 1994; Evans *et al.*, 1983; Hung *et al.*, 1998a, b). The exception in this analysis appears to be the dependence of taurocholate hepatic extraction on its fraction unbound in perfusate, presumably due to Bass-Pond effects (i.e. limitation in dissociation from proteins and diffusion to anion pumps) (Roberts *et al.*, 1990a).

The distributed model (Bass *et al.*, 1978; Bracken & Bass, 1979) represents the sinusoids of the liver as a very large ensemble of n functionally independent tubules over which there is a distribution of blood flow rates f_j and enzymatic elimination rates k_{ej}, for $j = 1, 2, \ldots, n$. (We consider only the limit of linear kinetics.) Assumptions are not needed in the model regarding either the constancy of cross-sectional areas of sinusoids, or their lengths, but for the present purposes it is convenient to simplify the modelling and suppose that each sinusoid has a constant area of cross-section, say a_j for the jth one, and that all the sinusoids have the same length L. The velocity of blood flow through the jth sinusoid is constant under the first of these further assumptions, at the value $v_j = f_j/a_j$. In terms of these modified assumptions and variables, and in the case that the distribution of elimination rates is narrow, the model enables steady-state hepatic extraction to be expressed in the form (Bass, 1980)

$$E = 1 - \exp(-R_N(1 + \tfrac{1}{2}\varepsilon^2 R_N^2)), \qquad (1)$$

with $\varepsilon^2 \ll 1$. Here R_N is the dimensionless "efficiency number" (Roberts & Rowland, 1986a) given by

$$R_N = \frac{k_{av}V}{F} = \frac{k_{av}L}{v_{av}}, \qquad (2)$$

and the dimensionless parameter ε^2 is given by

$$\varepsilon^2 = \frac{1}{F}\sum_{j=1}^{n} f_j \left(\frac{k_{ej}/k_{av}}{v_j/v_{av}}\right)^2 - 1. \qquad (3)$$

In eqn (2) and (3), $V = AL$ is the total liver (sinusoidal) volume, $F = \sum_{j=1}^{n} f_j$ is the total blood flow rate through the liver, $A = \sum_{i=1}^{n} a_i$ is the total area of cross-section of sinusoids, and v_{av}, k_{av} are average sinusoidal velocity and elimination rate, respectively, defined as

$$v_{av} = \frac{F}{A}, \qquad k_{av} = \frac{1}{A}\sum_{i=1}^{n} a_i k_{ei}. \qquad (4)$$

Roberts & Rowland (1985, 1986a) have shown that different forms of the dispersion model yield an equation similar in form to eqn (1), when $D_N \ll 1$ and a uniform enzyme distribution along sinusoids is assumed

$$E = 1 - \exp(-R_N(1 + D_N R_N^2)). \qquad (5)$$

In their study of the relationship between eqns (1) and (5), Bass *et al.* (1987) recognized that D_N used in equation (5), which we will refer to as D_N^E for clarity to represent the dispersion number defined by hepatic extraction, has two components, one due to heterogeneity of enzyme distribution in sinusoids, and the other due to vascular dispersion. They limited their considerations of enzyme heterogeneity to longitudinal distribution of enzymes. Roberts *et al.* (1988) recognized that transverse enzyme distribution was also important but limited their analysis to numerical simulations. Comparing the distributed model with the dispersion model for $D_N^E \ll 1$, Bass *et al.* (1987) suggested that the dispersion number for extracted solutes under the condition of steady-state input can be expressed explicitly as

$$D_N^E = D_N^a \frac{\overline{\rho^2}}{\bar{\rho}^2} = \tfrac{1}{2}\varepsilon^2. \qquad (6)$$

Here D_N^a is the *apparent* dispersion number, defined predominantly by the vascular dispersion (as can be obtained from the outflow profile of a non-extracted vascular reference after bolus administration), but also by the transverse enzyme heterogeneity. Also in eqn (6), $\rho(x)$ is a density function which describes distribution of enzyme along liver sinusoids (assumed by Bass *et al.* to be the same for all sinusoids), and

$$\bar{\rho} = \frac{1}{L}\int_0^L \rho(x)\,\mathrm{d}x, \qquad \overline{\rho^2} = \frac{1}{L}\int_0^L \rho(x)^2\,\mathrm{d}x. \qquad (7)$$

The interconnected-tubes model of hepatic elimination was developed, in part to provide a firmer physiological basis for the dispersion model, and to identify better the underlying determinants of the dispersion number in a mathematically precise and rigorous manner. Our initial analysis was limited to the construction of the model and its application to the specific case of the outflow concentration–time profile following bolus input (Anissimov *et al.*, 1997). It was shown that the zeroth-order approximation of the interconnected-tubes model is equivalent in form to the dispersion model and that the dispersion number can be explicitly expressed in terms of the determinants (1) heterogeneity of flow and (2) density of interconnections between sinusoids. It is important to note that the dispersion number as derived in this approximation does not involve heterogeneity of the elimination rates. The simplest form of the model, in comparison with experimental data, yielded an estimate of about ten interconnections, on average, between sinusoids.

We now consider the application of the interconnected-tubes model to the case of steady-state hepatic extraction. A specific goal is to clarify further the effects of enzyme heterogeneity and vascular dispersion on the dispersion number, complementing the study of Bass *et al.* (1987) and Roberts *et al.* (1988), and to do this in a more exact form using the mathematical analysis of the interconnected-tubes model. We shall show that the steady-state extraction of solutes is defined by a heterogeneity number as well as the efficiency number common to the various hepatic elimination models (Roberts & Rowland, 1986a). This heterogeneity number depends on both micromixing and transverse enzyme heterogeneity.

2. Brief Review: Interconnected-Tubes Model and Bolus Input

In order to describe both the sinusoidal structure of the liver, and the high degree of intermixing observed experimentally (Koo *et al.*, 1975), we modelled the elimination process in terms of a large number n of tubes acting in parallel, with various flow rates, with elimination within each tube, and with continuous interchange of substrate between tubes along their (common) length L (Anissimov *et al.*, 1997). For linear elimination kinetics, and under simplifying assumptions that cross-sectional areas, flow rates, elimination rate constants and coefficients describing exchange of solute between tubes are all constant along the tubes, the governing equations of the

interconnected-tubes model in matrix form are (Anissimov *et al.*, 1997)

$$\frac{\partial \mathbf{c}(x,t)}{\partial t} = -\mathbf{V}\frac{\partial \mathbf{c}(x,t)}{\partial x} - \mathbf{K}_e\mathbf{c}(x,t) + \mathbf{M}\mathbf{c}(x,t), \quad (8)$$

where t is the time, x the distance along the tubes from input at $x = 0$ to output at $x = L$, $\mathbf{c}(x,t) = (c_1(x,t), c_2(x,t), \ldots, c_n(x,t))^{\mathrm{T}}$ and $\mathbf{V} = \mathrm{diag}(v_i)$, $\mathbf{K}_e = \mathrm{diag}(k_{ei})$ and $\mathbf{M}$ are $n \times n$ matrices. For the i-th tube, $c_i(x,t)$ is the concentration, v_i is the (constant) velocity of blood flow, k_{ei} is the elimination rate constant and a_i is the (constant) cross-sectional area. In addition, $\mathbf{M} = \{m_{ij}\}$ is the matrix of coefficients of exchange between tubes, so that

$$m_{ij} = -\delta_{ij}\sum_{l=1}^{n} k_{il} + k_{ji}\frac{a_j}{a_i}, \quad i,j = 1, \ldots, n, \quad (9)$$

where k_{ij} is the constant coefficient of exchange from tube i to tube j. Due to physical constraints and consistency conditions on the system of interconnected tubes, the matrix $\mathbf{M}$ has the properties (Anissimov *et al.*, 1997)

$$\mathbf{a}^{\mathrm{T}}\mathbf{M} = 0, \quad \mathbf{M}\mathbf{e} = 0, \quad \mathrm{rank}(\mathbf{M}) = n - 1, \quad (10)$$

where $\mathbf{a} = (a_1, a_2, \ldots, a_n)^{\mathrm{T}}$ and $\mathbf{e} = (1, 1, \ldots, 1)^{\mathrm{T}}$.

It was assumed by Anissimov *et al.* (1997) that the coefficients of exchange k_{ij} are typically proportional to the average number of mixing sites N_{mix} along the sinusoids. More precisely, for each i,

$$T\max_{j\neq i}(k_{ij}) = \alpha N_{mix}, \quad (11)$$

where T is the average time of passage through the liver, and α is the coefficient of proportionality of order 1. If it is further assumed that $N_{max} \gg 1$ and therefore $T\max_{j\neq i} m_{ij} \gg 1$, then the solution of eqn (8) can be represented by the asymptotic series

$$\mathbf{c}(x,t) = \mathbf{e}k^{1/2}c_0(z,t) + \mathbf{c}^{(1)}(z,t) + k^{-1/2}\mathbf{c}^{(2)}(z,t) + \cdots, \quad (12)$$

where $k \gg 1$ is such that $k^{-1}T\max_{j\neq i} m_{ij} \sim 1$; $c_j^{(i)}(z,t) = O(1)$ for $z = (L/v_{av})O(1)$ and $c_j^{(i)}(z,t) \to 0$ for $|z| \gg T = L/v_{av}$ as $k \to \infty$, with

$$z = k^{1/2}(t - x/v_{av}), \quad (13)$$

and v_{av} the average sinusoidal velocity.

It was found in terms of the variables x and t, that the partial differential equation for c_0 is equivalent to the equation for the convection–dispersion model of hepatic elimination (Roberts & Rowland, 1985):

$$\frac{\partial c_0(x,t)}{\partial t} = -v_{av}\frac{\partial c_0(x,t)}{\partial x} + D\frac{\partial^2 c_0(x,t)}{\partial x^2} - k_{av}c_0(x,t). \quad (14)$$

The dispersion coefficient D is defined here in terms of parameters of the interconnected-tubes model (Anissimov *et al.*, 1997) by

$$D = \frac{\mathbf{a}^{\mathrm{T}}(\mathbf{V} - v_{av}\mathbf{I})\mathbf{w}^{(1)}}{Ak}v_{av}, \quad (15)$$

where $\mathbf{I}$ is a unit matrix and $\mathbf{w}^{(1)}$ is the solution of the linear algebraic equations

$$\mathbf{R}\mathbf{w}^{(1)} = \mathbf{b} = \mathbf{e} - \frac{\mathbf{v}}{v_{av}}, \quad \mathbf{e}^{\mathrm{T}}\cdot\mathbf{w}^{(1)} = \mathbf{0}, \quad (16)$$

and $\mathbf{R} = k^{-1}\mathbf{M}$, so that $\max_{i\neq j}(r_{ij}) \sim v_{av}/L$.

For the dimensionless dispersion number (Roberts & Rowland, 1986a), we then have

$$D_N = \frac{D}{Lv_{av}} = \frac{\mathbf{a}^{\mathrm{T}}(\mathbf{V} - v_{av}\mathbf{I})\mathbf{w}^{(1)}}{AkL}. \quad (17)$$

Although the heterogeneity of the elimination rates was modelled in eqn (8) by the term $\mathbf{K}_e$, its zeroth-order approximation (14) is independent of this heterogeneity. In particular, the dispersion number defined in eqn (17) is independent of the heterogeneity of the elimination rates and is determined only by the distribution of velocity, as defined by $\mathbf{v}$, and the strength and structure of interconnections as defined by $\mathbf{w}^{(1)}$. It is therefore reasonable to call D_N in eqn (17) the vascular

dispersion number D_N^V, as determined for example by the outflow concentration–time profile of a non-extracted vascular marker (Roberts *et al.*, 1990).

3. The Steady State

In the steady state, the time derivative approaches zero in eqn (8) and we have

$$\mathbf{V}\frac{\mathrm{d}\mathbf{c}}{\mathrm{d}x} = -\mathbf{K}_e\mathbf{c} + \mathbf{M}\mathbf{c}, \tag{18}$$

with the boundary condition

$$\mathbf{c}(0) = c_{in}\mathbf{e}, \tag{19}$$

where c_{in} is the input concentration of drug common to all sinusoids.

For two tubes ($n = 2$), eqn (18) is a pair of coupled linear first-order differential equations. Taking into account properties (10), the matrix $\mathbf{M}$ can be written as

$$\mathbf{M} = m\begin{pmatrix} -1 & 1 \\ \gamma & -\gamma \end{pmatrix}, \tag{20}$$

where $m = m_{12}$ and $\gamma = a_1/a_2$. The solution of eqn (18) with boundary condition (19) is, for $n = 2$,

$$c_1 = c_{in}\exp\left(\frac{xd}{2v_1v_2}\right) \times\left[\frac{d - 2v_2k_{e1}}{b}\sinh\left(\frac{xb}{2v_1v_2}\right) + \cosh\left(\frac{xb}{2v_1v_2}\right)\right],$$

$$c_2 = c_{in}\exp\left(\frac{xd}{2v_1v_2}\right) \times\left[\frac{d - 2v_1k_{e2}}{b}\sinh\left(\frac{xb}{2v_1v_2}\right) + \cosh\left(\frac{xb}{2v_1v_2}\right)\right], \tag{21}$$

where

$$b = \sqrt{(v_2(m + k_{e1}) - v_1(\gamma m + k_{e2}))^2 + 4\gamma k^2 v_1 v_2}$$

and

$$d = v_2(m + k_{e1}) + v_1(\gamma m + k_{e2}).$$

For the case of strong interconnection between tubes ($m = kr$, with $k \gg 1$ and $r \sim v_{av}/L$), one can easily obtain the first-order approximations to c_1 and c_2 for large k:

$$c_1(x) = c_{in}\exp\left(-\frac{k_{av}}{v_{av}}x\right)\left[1 + \frac{1}{k}\frac{(k_{e2}v_1 - k_{e1}v_2)[x\gamma(k_{e2}v_1 - k_{e1}v_2) + v_2(\gamma v_1 + v_2)]}{r(\gamma v_1 + v_2)^3}\right],$$

$$c_2(x) = c_{in}\exp\left(-\frac{k_{av}}{v_{av}}x\right)\left[1 + \frac{1}{k}\frac{(k_{e1}v_2 - k_{e2}v_1)[x\gamma(k_{e1}v_2 - k_{e2}v_1) + v_1\gamma(\gamma v_1 + v_2)]}{r(\gamma v_1 + v_2)^3}\right], \tag{22}$$

We note that these asymptotic formulae are correct only if $\exp(-krx(\gamma/v_2 + 1/v_1))$ can be neglected, that is for not very small values of x.

For the flow-weighted output concentration c_{out} we have

$$c_{out} = \frac{a_1v_1c_1(L) + a_2v_2c_2(L)}{a_1v_1 + a_2v_2} = \frac{\gamma v_1c_1(L) + v_2c_2(L)}{\gamma v_1 + v_2},$$

and substituting c_1 and c_2 from eqn (22) we get

$$c_{out} = c_{in}\exp\left(-\frac{k_{av}}{v_{av}}L\right) \times\left[1 + \frac{1}{k}\frac{\gamma L(k_{e1}v_2 - k_{e2}v_1)^2}{(\gamma v_1 + v_2)^3}\right]. \tag{23}$$

The form of the asymptotic series expansion for large k, as follows from eqn (22), is

$$c_i = f_0(x) + k^{-1} f_i^{(1)}(x) + k^{-2} f_i^{(2)}(x) + \cdots, \quad i = 1, 2, \tag{24}$$

where f_0 and $f_i^{(j)}$ are some functions of x which are $O(1)$ as $k \to \infty$. Note that this form is different from eqn (12), the form obtained for bolus input.

Having the exact solution, and the approximation to it for large k, in the case of two tubes, we can now analyse the steady-state case for a system of n interconnected tubes. As in the time-dependent case, we consider $m_{ij} = kr_{ij}$ with $k \gg 1$, so that $\max_{i \neq j}(r_{ij}) \sim v_{av}/L$. Given the form of approximation (24) for large k in the case of two tubes, it is reasonable to expect for $\mathbf{c}$ an asymptotic series expansion of the form

$$\mathbf{c}(x) = \mathbf{e}c_0(x) + \frac{1}{k}\mathbf{c}^{(1)}(x) + \frac{1}{k^2}\mathbf{c}^{(2)}(x) + \cdots, \tag{25}$$

which should not be valid for very small values of x. We will represent $\mathbf{c}^{(i)}$ by two components, one parallel to and one orthogonal to $\mathbf{e}$,

$$\mathbf{c}^{(i)}(x) = \mathbf{e}c_0^{(i)}(x) + \mathbf{d}^{(i)}(x), \tag{26}$$

and substitute eqn (25) into eqn (18), with $\mathbf{M} = k\mathbf{R}$. Grouping terms with the same power of k yields

$$k^0\text{:}\ \mathbf{v}\frac{\mathrm{d}}{\mathrm{d}x}c_0(x) = -\mathbf{k}_e c_0(x) + \mathbf{R}\mathbf{d}^{(1)}(x), \tag{27}$$

$$k^{-1}\text{:}\ \mathbf{v}\frac{\mathrm{d}}{\mathrm{d}x}c_0^{(1)}(x) + \mathbf{V}\frac{\mathrm{d}}{\mathrm{d}x}\mathbf{d}^{(1)}(x) = -\mathbf{k}_e c_0^{(1)}(x) - \mathbf{K}_e\mathbf{d}^{(1)}(x) + \mathbf{R}\mathbf{d}^{(2)}(x), \tag{28}$$

$$k^{-i}\text{:}\ \mathbf{v}\frac{\mathrm{d}}{\mathrm{d}x}c_0^{(i)}(x) + \mathbf{V}\frac{\mathrm{d}}{\mathrm{d}x}\mathbf{d}^{(i)}(x) = -\mathbf{k}_e c_0^{(i)}(x) - \mathbf{K}_e\mathbf{d}^{(i)}(x) + \mathbf{R}\mathbf{d}^{(i+1)}(x), \tag{29}$$

where $i \geqslant 2$, $\mathbf{v}^{\mathrm{T}} = (\mathbf{V}\mathbf{e})^{\mathrm{T}} = (v_1, v_2, \ldots, v_n)$ and $k_e^{\mathrm{T}} = (\mathbf{K}_e\mathbf{e})^{\mathrm{T}} = (k_{e1}, k_{e2}, \ldots, k_{en})$. We now multiply eqn (27) by $\mathbf{a}^{\mathrm{T}}$ on the left, and using $\mathbf{a}^{\mathrm{T}}\mathbf{R} = 0$ we get

$$\frac{\mathrm{d}c_0(x)}{\mathrm{d}x} = -\frac{\mathbf{a}^{\mathrm{T}}\mathbf{k}_e}{\mathbf{a}^{\mathrm{T}}\mathbf{v}}c_0(x) = -\frac{k_{av}}{v_{av}}c_0(x), \tag{30}$$

as we have $\mathbf{a}^{\mathrm{T}}\mathbf{v} = Av_{av}$ and $\mathbf{a}^{\mathrm{T}}\mathbf{k}_e = Ak_{av}$. Thus, the zeroth-order approximation for the steady-state leads to a single tube equation, or the tube model (Bass *et al.*, 1976), with the elimination rate k_{av} and the flow velocity v_{av}. The solution of eqn (30) is

$$c_0(x) = C_1 \exp\left(-\frac{k_{av}}{v_{av}}x\right), \tag{31}$$

where C_1 is an arbitrary constant. To fix C_1, we cannot directly use the boundary condition (19), as the asymptotic expansion (25) and thus the differential equation (30) for $c_0(x)$ is not valid for very small x. Instead, we will later use a comparison with the two-tubes case to determine C_1.

Having substituted $c_0(x)$ from eqn (31) into eqn (27), we get

$$\mathbf{R}\mathbf{d}^{(1)}(x) = k_{av}\left(\frac{\mathbf{k}_e}{k_{av}} - \frac{\mathbf{v}}{v_{av}}\right)c_0(x) \tag{32}$$

or

$$\mathbf{d}^{(1)}(x) = k_{av}\mathbf{u}^{(1)}c_0(x), \tag{33}$$

where $\mathbf{u}^{(1)}$ is the solution of the linear algebraic equations

$$\mathbf{R}\mathbf{u}^{(1)} = \left(\frac{\mathbf{k}_e}{k_{av}} - \frac{\mathbf{v}}{v_{av}}\right), \quad \mathbf{e}^{\mathrm{T}}\mathbf{u}^{(1)} = 0. \tag{34}$$

The solution of eqn (34) exists and is unique because $\mathbf{R}$ shares the property (10) with $\mathbf{M}$.

We note that in the case of uniform elimination rates, where $\mathbf{k}_e = k_{av}\mathbf{e}$, eqn (34) can be written as

$$\mathbf{R}\mathbf{u}^{(1)} = \left(\mathbf{e} - \frac{\mathbf{v}}{v_{av}}\right), \quad \mathbf{e}^{\mathrm{T}}\mathbf{u}^{(1)} = 0. \tag{35}$$

Comparing this equation with the definition of $\mathbf{w}^{(1)}$ in eqn (16), it is easily seen that

$$\mathbf{u}^{(1)} = \mathbf{w}^{(1)}. \tag{36}$$

To find $c_0^{(1)}(x)$, we multiply eqn (28) by $\mathbf{a}^T$ from the left, use $\mathbf{a}^T\mathbf{R} = 0$ again, and substitute $\mathbf{d}^{(1)}(x)$ in the form (33). The equation for $c_0^{(1)}(x)$ is now

$$Av_{av}\frac{dc_0^{(1)}(x)}{dx} + Ak_{av}c_0^{(1)}(x) = k_{av}\left(\mathbf{a}^T\mathbf{V}\mathbf{u}^{(1)}\frac{k_{av}}{v_{av}} - \mathbf{a}^T\mathbf{K}_e\mathbf{u}^{(1)}\right)c_0(x). \quad (37)$$

The general solution of this differential equation is

$$c_0^{(1)}(x) = C_1(C_2 + Bx)\exp\left(-\frac{k_{av}}{v_{av}}x\right), \quad (38)$$

where

$$B = \frac{k_{av}^2}{Av_{av}^2}\mathbf{a}^T\left(\mathbf{V} - v_{av}\frac{\mathbf{K}_e}{k_{av}}\right)\mathbf{u}^{(1)}, \quad (39)$$

and C_2 is another constant.

Thus, the first-order approximation is

$$\mathbf{c}(x) = C_1\exp\left(-\frac{k_{av}}{v_{av}}x\right) \times\left(\mathbf{e} + \frac{k_{av}}{k}\mathbf{u}^{(1)} + \frac{1}{k}\mathbf{e}\,(C_2 + Bx)\right). \quad (40)$$

The corresponding approximation to the flow-weighted output concentration is

$$c_{out} = \frac{\mathbf{a}^T\mathbf{V}\mathbf{c}(L)}{\mathbf{a}^T\mathbf{v}} = C_1\exp\left(-\frac{k_{av}}{v_{av}}x\right)\left(1 + \frac{1}{k}BL + \frac{1}{k}\left(C_2 + \frac{k_{av}\mathbf{a}^T\mathbf{V}\mathbf{u}^{(1)}}{\mathbf{a}^T\mathbf{v}}\right)\right). \quad (41)$$

To find the constants C_1 and C_2 we may use a comparison with the two-tubes case. Comparing eqn (23) with eqn (41) we see that $C_1 = c_{in}$. In eqn (23), the term of order k^{-1} is proportional to L, so that in eqn (41) the third term in parentheses must be zero, that is

$$C_2 = -\frac{k_{av}\mathbf{a}^T\mathbf{V}\mathbf{u}^{(1)}}{\mathbf{a}^T\mathbf{v}}, \quad (42)$$

and for the output concentration we have

$$c_{out} = c_{in}(1 + H_N R_N^2)e^{-R_N}, \quad (43)$$

where

$$H_N = \frac{\mathbf{a}^T(\mathbf{V} - v_{av}\mathbf{K}_e/k_{av})\mathbf{u}^{(1)}}{AkL}, \quad (44)$$

which we call the heterogeneity number, and R_N is the efficiency number eqn (2). It is clear from definition (44) that H_N takes into account not only heterogeneity of flow rates, as described by D_N^V, but variations of enzyme activity as well.

When elimination rates in all tubes are the same, then $\mathbf{u}^{(1)} = \mathbf{w}^{(1)}$ as in eqn (36), and $\mathbf{K}_e/k_{av} = \mathbf{I}$. Hence, defining D_N^V by eqn (17), and H_N by eqn (44), it is evident that $H_N = D_N^V$ in this case. It is obvious from eqn (44) that for the case of full correlation between $\{k_{ej}\}$ and $\{v_j\}$, that is $(k_{ej} - k_{av})/k_{av} = (v_j - v_{av})/v_{av}$, we have $H_N = 0$ and therefore $H_N < D_N^V$. For full anti-correlation (also called full inverse correlation or full negative correlation), when $(k_{ej} - k_{av})/k_{av} = -(v_j - v_{av})/v_{av}$ (for positiveness of $\{k_{ej}\}$ and $\{v_j\}$ in this case we need to limit distributions so that $\max k_{ei} \leqslant 2k_{av}$ and $\max v_i \leqslant 2v_{av}$) comparison of eqns (44) and (17) gives instead $H_N = 4D_N^V$ and therefore $H_N > D_N^V$. This analysis gives some idea as to what the dependence of H_N on relative distribution of velocities and elimination rates could be for an arbitrary system of interconnected tubes.

We note that the heterogeneity of enzyme distribution described by Bass *et al.* (1987) [the term $\overline{\rho^2}/\bar{\rho}^2$ in eqn (6)] is due to the distribution of enzyme along sinusoids and is different from the heterogeneity of elimination rates considered in this paper, which is due to the different enzyme activities in different sinusoids (transverse distribution of enzyme). In fact, because we assumed, for simplicity, that each k_{ei} is constant along the

corresponding tube, it follows that ρ is independent of x in our analysis, and hence $\overline{\rho^2}/\bar{\rho}^2 = 1$ in eqn (6).

4. The Dispersion and Heterogeneity Numbers

Equations (17) and (44) give a quantitative definition for the dispersion and heterogeneity numbers, previously described only qualitatively by Roberts & Rowland (1986a). Unfortunately, these equations involve vectors $\mathbf{w}^{(1)}$ and $\mathbf{u}^{(1)}$ which are solutions to linear algebraic equations (16) and (34), and cannot be expressed in a simple form for a general system of interconnected tubes. In order to throw some light on these expressions, we will consider a few specific examples where the expressions for the vectors $\mathbf{w}^{(1)}$ and $\mathbf{u}^{(1)}$, and thus for D_N^V and H_N, are simple.

As a simple example of a model n-tube system, we consider first the case of n tubes with equal cross-sectional areas ($a_i = A/n$) and interconnections between neighbouring tubes, so that each tube is connected to two tubes, except the first and last which are connected to only one neighbouring tube. We will consider equal rates of exchange between tubes. The coefficient of interconnections in this case is

$$k_{ij} = kr(\delta_{ij-1} + \delta_{i-1j}), \quad i \neq j,$$

and the matrix $\mathbf{R}$ is

$$\mathbf{R} = r\begin{pmatrix} -1 & 1 & 0 & \cdots & & & 0 \\ 1 & -2 & 1 & 0 & \cdots & & 0 \\ 0 & 1 & -2 & 1 & 0 & \cdots & 0 \\ \vdots & & & & & & \vdots \\ 0 & \cdots & & & 0 & 1 & -1 \end{pmatrix}$$

Using this matrix to solve eqns (34) yields for $\mathbf{u}^{(1)}$:

$$u_i^{(1)} = \frac{1}{r}\left(\sum_{j=1}^{i-1}(i-j)b_j - \frac{1}{n}\sum_{k=2}^{n}\sum_{j=1}^{k-1}(k-j)b_j\right), \tag{45}$$

where $b_i = k_{ei}/k_{av} - v_i/v_{av}$.

As all tubes have equal cross-sectional areas, then $\mathbf{a}^{\mathrm{T}} = A\mathbf{e}^{\mathrm{T}}/n$, and

$$\mathbf{a}^{\mathrm{T}}(\mathbf{V} - v_{av}\mathbf{K}_e/k_{av}) = \frac{A}{n}(\mathbf{v}^{\mathrm{T}} - \mathbf{k}_e^{\mathrm{T}}v_{av}/k_{av}) = \frac{Av_{av}}{n}\mathbf{b}^{\mathrm{T}},$$

so that formula (44) for H_N simplifies to

$$H_N = \frac{v_{av}}{nkL}\mathbf{b}^{\mathrm{T}}\mathbf{u}. \tag{46}$$

Using eqn (45) for $\mathbf{u}^{(1)}$ and taking into account that $\mathbf{b}^{\mathrm{T}}\mathbf{e} = 0$, we get

$$H_N = -\frac{v_{av}}{nkrL}\sum_{i=2}^{n}\sum_{j=1}^{i-1}(i-j)b_jb_i.$$

This equation can be rewritten as

$$H_N = \frac{1}{nkrLv_{av}k_{av}^2}\sum_{i=2}^{n}\left(\sum_{j=1}^{i-1}(k_{ej}v_{av} - v_jk_{av})\right)^2. \tag{47}$$

To get an expression for D_N^V, we simply need to take $\mathbf{k}_e/k_{av} = \mathbf{e}$ (as for the uniform elimination rates $D_N^V = H_N$) in eqn (47), thus

$$D_N^V = \frac{1}{nkrLv_{av}}\sum_{i=2}^{n}\left(\sum_{j=1}^{i-1}(v_{av} - v_j)\right)^2. \tag{48}$$

It is easy to see now that D_N^V and H_N are positive for any n in this model system. Note as well that eqn (47) for H_N can be written in a form somewhat similar to that of ε^2 in eqn (3). Indeed, as $av_j = f_j$, $anv_{av} = F$ and $krT = krL/v_{av} = \alpha N_{mix}$ as in eqn (11), then eqn (47) can be modified to

$$H_N = \frac{1}{F^2\alpha N_{mix}}\sum_{i=2}^{n}\left(\sum_{j=1}^{i-1}f_j\left(\frac{k_{ej}/k_{av}}{v_j/v_{av}} - 1\right)\right)^2.$$

Another model system which we can analyse consists of n tubes all connected to each other. Cross-sectional areas of tubes and rates of exchange between tubes are again assumed equal.

Coefficients of exchange will be $k_{ij} = kr$, $i \neq j$, and in this case the matrix $\mathbf{R}$ is

$$\mathbf{R} = \begin{pmatrix} -(n-1) & 1 & \cdots & 1 \\ 1 & -(n-1) & 1 & \cdots & 1 \\ \vdots & & & & \vdots \\ 1 & \cdots & & 1 & -(n-1) \end{pmatrix}.$$

Using this matrix we get for H_N,

$$H_N = \frac{1}{n^2 k L v_{av} k_{av}^2} \sum_{i=1}^{n} (k_{ej} v_{av} - v_j k_{av})^2. \quad (49)$$

Taking $\mathbf{k}_e / k_{av} = \mathbf{e}$ we get for D_N^V

$$D_N^V = \frac{1}{n^2 k L v_{av}} \sum_{i=1}^{n} (v_{av} - v_j)^2. \quad (50)$$

Once again, these expressions for H_N and D_N^V are obviously positive.

Equations (47)–(50) show how H_N and D_N^V depend on the distribution of velocities and elimination rates for the two simple model systems of interconnected tubes considered in this section.

While it is not yet proved that H_N and D_N^V defined in eqns (44) and (17) are positive for any system of interconnected tubes, the two examples considered here give some ground to believe that this is true in general, as would be expected intuitively.

It is interesting to note that in terms of interconnections between tubes, the first model system considered in this section represents 'minimal' coupling and the second 'maximal' coupling. In the first case, each tube is connected only to neighbouring tubes, whereas in the second case all the tubes are connected with each other. Comparing expressions for the dispersion number for the first case, eqns (47) and (48), with expressions for the second case, eqns (49) and (50), one can see that the dispersion number for the maximal coupling is much less than for the minimal coupling. In fact, $D_{N1}^V \geqslant n D_{N2}^V$, where D_{N1}^V and D_{N2}^V are dispersion numbers for the first and the second cases, respectively. Together with D_N^V being proportional to $1/k$, this leads to the conclusion that the heterogeneity of transit times through the liver decreases with increase of coupling between sinusoids. It is not clear, yet, to what degree this conclusion is due to the particular way of modelling of interconnections between the sinusoids in eqn (8). We hope to verify this dependence of the heterogeneity of transit times on the strength of coupling in future by directly modelling discrete systems of interconnected tubes on the computer.

It was shown previously (Anissimov *et al.*, 1997) that for two interconnected sinusoids, the coefficient of interconnection k_{12} can be approximated using

$$k_{12} T = k_{12} L / v_{av} \approx \alpha N, \quad (51)$$

where $\alpha \approx 0.7$ and N is the number of interconnections between two sinusoids. Extending eqn (51) to a system of n interconnected tubes we have

$$k_{ij} T \approx \alpha N_{ij}, \quad (52)$$

where N_{ij} is the number of interconnections between the i-th and j-th sinusoids. Summation of eqn (52) with respect to index j and then averaging for an entire system of n tubes yields

$$\overline{\sum_{j \neq i} k_{ij} T} \approx \alpha N_{mix}, \quad (53)$$

where N_{mix} is the average number of sinusoids connected to any one sinusoid. Applying eqn (53) to the model systems considered in this section it is possible to approximate krT:

$$krT \approx \alpha N_{mix} \frac{n}{2(n-1)} \quad (54)$$

for the model system with 'minimal' coupling, and

$$krT \approx \alpha N_{mix} \frac{1}{n-1} \quad (55)$$

for the model system with 'maximal' coupling.

Using eqns (54) and (55), eqns (48) and (50) can be written for the two-model systems as

$$D_{N1}^V \approx \frac{1}{\alpha N_{mix}} \frac{2(n-1)}{n^2} \sum_{i=2}^{n} \left(\sum_{j=1}^{i-1} \left(1 - \frac{v_j}{v_{av}} \right) \right)^2, \quad (56)$$

$$D_{N2}^V \approx \frac{1}{\alpha N_{mix}} \frac{n-1}{n^2} \sum_{i=1}^{n} \left(1 - \frac{v_j}{v_{av}} \right)^2. \quad (57)$$

These formulae give some insight into the way in which the distribution of velocities, and the nature of interconnections, contribute to the dispersion number. It is possible to approximate D_N^V now if the distribution of v_j values and the average number of interconnections per sinusoid N_{mix} are known. We note though, that due to the simplified way of modelling interconnections in the model systems presented here, the number n in eqns (56) and (57) should not be regarded strictly as the number of sinusoids in the entire liver. It may be interpreted rather as the number of sinusoids which are strongly interconnected with each other.

The actual distribution of the velocity of flow in sinusoids is shown in Fig. 1; it was obtained from the data of Koo *et al.* (1975). The frequency distributions of the direct, interconnected and branching sinusoids were summed to get the frequency distribution for all sinusoids. Grouping all sinusoids into n sets with similar velocities and equal numbers of sinusoids in each set, we find v_j, with $j = 1, \ldots, n$, as an average velocity of the j-th set. Using these v_j in eqns (56) and (57), together with $\alpha = 0.7$ and $N_{mix} = 5$ (Bass *et al.*, 1987), yields for D_N^V:

n	2	3	5	10	100
D_{N1}^V	0.0294	0.1001	0.3450	1.5727	173.56
D_{N2}^V	0.0294	0.0514	0.0712	0.0882	0.1138

where D_{N1}^V and D_{N2}^V are dispersion numbers for model systems with "minimal" and "maximal" couplings, respectively. It is interesting that the dispersion number is typically greater for "minimal" than "maximal" coupling. For $n = 5$, the values $D_{N1}^V = 0.3450$ and $D_{N2}^V = 0.0712$ provide

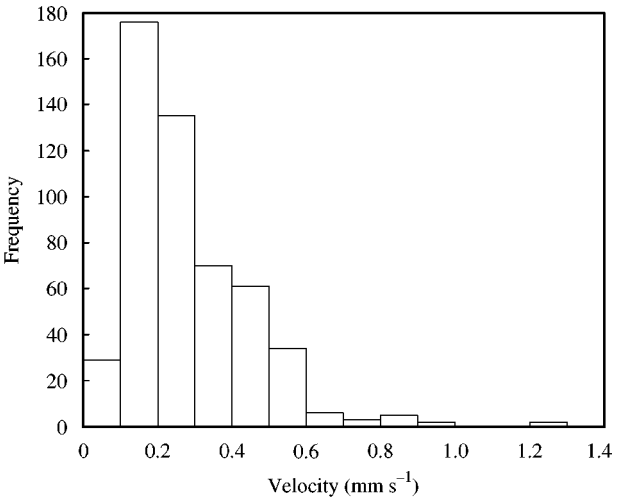

FIG. 1. Frequency distribution of the velocity of flow in sinusoids (adapted from Koo *et al.*, 1975).

reasonable upper and lower bounds, respectively, for experimental values of the dispersion number (Roberts *et al.*, 1988). The large values of D_{N1}^V for the model system with "minimal" coupling and with $n \geqslant 10$, are unrealistic, and probably arise because k is not big enough to validate the analysis in those cases. We recall that eqns (56) and (57) are only valid for $k \gg 1$; in fact k is only about 3 for $N_{mix} = 5$.

It should be noted that the analysis of the value of the dispersion number presented here is very approximate. Further, using simplified model systems, we have not taken into account the heterogeneity of lengths of liver sinusoids. This heterogeneity might increase the effective heterogeneity of velocities, thus increasing the value of D_N^V.

5. Discussion

Further analyses of the interconnected-tubes model has been presented in this work. The approximation to the interconnected-tubes model for a large number of mixing sites ($N_{mix} \gg 1$) for the steady-state input has been developed and results compared with that of bolus input.

It is interesting to compare the first-order approximation to the interconnected-tubes model, given by eqn (43), with the result for the dispersion model, where D_N for consistency must be

taken as a small parameter ($D_N \ll 1$), because we have $D = O(k^{-1})$. Recognizing that $c_{out} = c_{in}(1 - E)$, and keeping only terms to the order of D_N in the approximation $D_N \ll 1$ of the dispersion model (5), yields

$$c_{out} \approx c_{in}(1 + D_N^E R_N^2)\mathrm{e}^{-R_N}, \qquad (58)$$

where D_N^E is the dispersion number for extracted solutes in the steady state, as defined in eqn (6). This prediction of the dispersion model is equivalent to that of the interconnected-tubes model, as in eqn (43) with $H_N = D_N^E$. In the case of arbitrary elimination rates, $H_N \neq D_N^V$, and therefore $D_N^V \neq D_N^E$. We see that, because of its approximate nature, the dispersion model equation (14) cannot be applied to the steady state and bolus input cases with the same dispersion number, when substantial enzyme heterogeneity exists.

Owing to the inclusion of the intermixing terms in equations for the interconnected-tubes model, H_N depends now not only on distributions of k_{ei} and v_i, as does ε^2, but also on the extent and the structure of the intermixing between the sinusoids. In eqn (44), the parameters k and $\mathbf{u}^{(1)}$ (which itself depends on $\mathbf{R}$) determine this dependence of H_N on intermixing.

An important theoretical result for the distributed model is its prediction that ε^2 is independent of small changes in the hepatic blood flow F. We shall now examine whether H_N depends on F. Suppose that a change of F is small enough not to change the shape of the distribution of velocities v_i in sinusoids; more precisely, suppose that $v_i \propto F$ for all i. Then, for the average velocity (4) we also have $v_{av} \propto F$. It was argued already in eqn (11) that $T \max_{j \neq i} k_{ij} = \alpha N_{mix}$, and with N_{mix} unchanged and $T = L/v_{av} \propto 1/F$, we have for the coefficient of exchange, $k_{ij} \propto F$. Then $r_{ij} \propto F$, as we expect k to be flow-independent. Using eqn (34), we now obtain $\mathbf{u}^{(1)} \propto 1/F$. Finally, using eqn (44) it is easy to see that H_N is independent of small changes of the hepatic blood flow. This result is in agreement with the experimental data for the steady state (Bass, 1980). Similar analysis for the vascular dispersion number shows that D_N^V is also independent of small changes of the hepatic blood flow, in agreement with experiments (Roberts *et al.*, 1990a).

The zeroth-order approximation to the interconnected-tubes model for bolus input results in the convection–dispersion–reaction equation (14) (Anissimov *et al.*, 1997). In contrast, for the steady state, the zeroth-order approximation gives the convection equation (30). The understanding of this apparently contradictory result comes with the analysis of the order of magnitude of each term in eqns (8) and (18). In eqn (8), the derivatives $\partial\mathbf{c}/\partial x$ and $\partial\mathbf{c}/\partial t$ are of a higher order in the expansion parameter of k than is $\mathbf{c}$, because $\mathbf{c}(x, t)$ is sharply peaked at $x = tv_{av}$. However in eqn (18), $\mathrm{d}\mathbf{c}/\mathrm{d}x$ is of the same order of magnitude as $\mathbf{c}$. As $D = O(k^{-1})$ according to eqn (15), so $D_N^V \ll 1$, and the term $Dd^2c_0/\mathrm{d}x^2$ disappears in the zeroth-order approximation of eqn (14) as a steady-state expression. Hence, eqn (14) is equivalent to eqn (30) in the steady-state case.

The comparison of the steady-state case and the case of bolus input leads to the realisation that the dispersion model, when viewed as an approximation to the interconnected-tubes model, does not comply with familiar notions of linear modelling when enzyme heterogeneity exists. We would normally expect that the response of the system to the unit impulse input (Green's function) defines the response for any input. However, eqn (43), the approximation to the interconnected-tubes model for the steady-state input, is not the same as the corresponding equation (58) for the dispersion model when $H_N \neq D_N^V$. The mathematical reason for this discrepancy being the approximate nature of the dispersion model when viewed as a limiting case of the interconnected-tubes model. This inability to use the Green's function for the dispersion model will be particularly troublesome if the input is an arbitrary function of time and if enzyme heterogeneity exists. We emphasize that when there is no distribution of elimination rates ($\mathbf{k}_e = \mathbf{e}k_e$) over sinusoids, or its distribution is far narrower than the distribution of transit times (which could be the case for many drugs), the discrepancy between H_N and D_N^V disappears, and this difficulty along with it.

It should be emphasized that the present comparison of the interconnected-tubes model with the dispersion model is limited to the specific case where $D_N^E \ll 1$. The extent to which the results can be applied to hepatic elimination is uncertain

since physiologically observed D_N^V values range from 0.2 to 0.4.

Just as the Goresky and convection–dispersion models have two-compartment extensions (Goresky *et al*., 1973; Roberts *et al*., 1988; Yano *et al*., 1989), so also the interconnected-tubes model could be extended by assuming two compartments within each tube: one cellular, with no longitudinal flow through it, and one vascular, with convective (longitudinal) transport. Exchange of drug between vascular and cellular compartments could be modelled with rates $k1_i$ and $k2_i$, and elimination from cellular compartments with rates k_{eci}. The vascular volume of distribution, as defined by the tube cross-section a_i, is known to be dependent on the size of the "substance" being examined. Thus, erythrocytes are restricted to the sinusoidal space while albumin has a lower vascular volume of distribution than the smaller sucrose and sodium (Goresky, 1963). Distribution volumes which extend into the cellular compartments are often defined by the physiological distribution space for the unbound solute together with the relative binding in the vascular and cellular spaces (Weiss & Roberts, 1996). Such refinements to the interconnected-tubes model can be expected to yield similar outcomes, in terms of transit times and outflow concentration–time profiles, that predicted by the Goresky and convection–dispersion models.

Analysis of an interconnected-tubes model which includes heterogeneity of enzyme distribution in a two-compartment representation is considered to be possible but requires more complex mathematics. Other extensions can also be envisaged. Intrahepatic shunting, caused by cirrhosis for example, can also be modelled by assuming that a subset of affected sinusoids have elimination rates much lower than those of unaffected sinusoids. One implication of such a model of shunting will be larger heterogeneity of elimination rates, and therefore a larger discrepancy between D_N^V and H_N. In this work, the analysis of the interconnected-tubes model has been limited to a simple case where both elimination rates k_{ei}, and coefficients of exchange k_{ij}, are constant along tubes. These assumptions were made to allow some progress with the mathematics. For a numerical treatment of the model, these simplifying assumptions can be relaxed, and more complex phenomena analysed. For example, it is then possible to model localised "zones" of elimination in the liver, allowing preferential elimination or metabolism in, say, the periportal zone of the liver acinus (Gumucio & Miller, 1981). It is only necessary to allow $k_{ei}(x)$ to become functions which decrease with increasing x, perhaps in discrete steps. It has been argued that there are more interconnections between the sinusoids in the periportal region as compared to the previous region of acini (Gumucio, 1983). This structure of liver acini can also be modelled, by allowing the $k_{ij}(x)$ to decrease as x increases.

Finally, we want to emphasize that mathematically, the convection–dispersion and distributed models can both be regarded as the limiting cases of the interconnected-tubes model. If there are few interconnections between tubes (that is $k \approx 0$), then eqn (8) is essentially identical to the defining equations of the distributed model, whereas for $k \gg 1$, the zeroth-order approximation to eqn (8) is the defining partial differential equation of the convection–dispersion model, as we have shown. This ensures that the interconnected-tubes model can be made consistent with a wide range of experimental situations already successfully described using either the convection–dispersion or distributed models (Luxon & Weisiger, 1993; Rivory *et al*., 1992; Roberts *et al*., 1990a, b; Yano *et al*., 1989; Bass *et al*., 1987). It is an important challenge to devise new experiments which can discriminate among various models more effectively and in particular provide experimental checks of the more detailed interconnected-tubes model, and estimates of its parameters.

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REFERENCES

ANISSIMOV, Y. G., BRACKEN, A. J. & ROBERTS, M. S. (1997). Interconnected-tubes model of hepatic elimination. *J. theor. Biol.* **188,** 89–101.

BASS, L. (1980). Flow dependence of first-order uptake of substances by heterogeneous perfused organs. *J. theor. Biol.* **86,** 365–376.

BASS, L. & KEIDING, S. (1988). Physiologically based models and strategic experiments in hepatic pharmacology. *Biochem. Pharmacol.* **37,** 1425–1431.

BASS, L., KEIDING, S., WINKLER, K. & TYGSTRUP, N. (1976). Enzymatic elimination of substrates flowing through the intact liver. *J. theor. Biol.* **61,** 393–409.

BASS, L., ROBERTS, M. S. & ROBINSON, P. J. (1987). On the relation between extended forms of the sinusoidal perfusion and of the convection–dispersion models of hepatic elimination. *J. theor. Biol.* **126,** 457–482.

BASS, L., ROBINSON, P. J. & BRACKEN, A. J. (1978). Hepatic elimination of flowing substrates: the distributed model. *J. theor. Biol.* **72,** 161–187.

BRACKEN, A. J. & BASS, L. (1979). Statistical mechanics of hepatic elimination. *Math. Biosci.* **44,** 97–120.

CHOU, C. H., EVANS, A. M., FORNASINI, G. & ROWLAND, M. (1993). Relationship between lipophilicity and hepatic dispersion and distribution for a homologous series of barbiturates in the isolated perfused *in situ* rat liver. *Drug Metab. Disp.* **21,** 933–938.

EVANS, A. M., HUSSEIN, Z. & ROWLAND, M. (1993). Influence of albumin on the distribution and elimination kinetics of diclofenac in the isolated perfused rat liver: analysis by the impulse-response technique and the dispersion model. *J. Pharm. Sci.* **82,** 421–428.

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

GORESKY, C. A., BACH, G. G. & NADEAU, B. E. (1973). On the uptake of materials by the intact liver: the transport and net removal of galactose. *J. Clin. Invest.* **52,** 991–1009.

GUMUCIO, J. J. (1983). Functional and anatomical heterogeneity in the liver acinus: impact on transport. *Am. J. Physiol.* **244,** G578–G582.

GUMUCIO, J. J. & MILLER, D. L. (1981). Functional implications of liver cell heterogeneity. *Gastroenterology*, **80,** 393–403.

HUNG, D. Y., MELLICK, G. D., ANISSIMOV, Y. G., WEISS, M. & ROBERTS, M. S. (1998a). Hepatic structure-pharmacokinetic relationship: the hepatic disposition and metabolite kinetics of a homologous series of O-acyl derivatives of salicylic acid. *Br. J. Pharmacol.* **124,** 1475–1483.

HUNG, D. Y., MELLICK, G. D., ANISSIMOV, Y. G., WEISS, M. & ROBERTS, M. S. (1998b). Hepatic disposition and metabolite kinetics of a homologous series of a homologous series of diflunisal esters. *J. Pharm. Sci.* **87,** 943–951.

HUSSEIN, Z., MCLACHLAN, A. J. & ROWLAND, M. (1994). The distribution kinetics of salicylic acid in the isolated perfused rat liver assessed using moment analysis and the two-compartment axial dispersion model. *Pharm. Res.* **11,** 1337–1345.

IWATSUBO, T., SUZUKI, H., SHIMADA, N., CHIBA, K., ISHIZAKI, T., GREEN, C. E., TYSON, C. A., YOKOI, T., KAMATAKI, T. & SUGIYAMA, Y. (1997). Prediction of *in vivo* hepatic metabolic clearance of YM796 from *in vitro* data by use of human liver microsomes and recombinant P-450 isozymes. *J. Pharmacol. Exp. Ther.* **282,** 909–919.

KOO, A., LIANG, I. Y. S. & CHENG, K. K. (1975). The terminal hepatic microcirculation in the rat. *Quart. J. Exp. Physiol.* **60,** 261–266.

LUXON, B. A. & WEISIGER, R. A. (1993). Extending the multiple indicator dilution method to include slow intracellular diffusion. *Math. Biosci.* **113,** 211–230.

RIVORY, L. P., ROBERTS, M. S. & POND, S. M. (1992). Axial tissue diffusion can account for the disparity between current models of hepatic elimination for lipophillic drugs. *J. Pharmacokin Biopharm.* **20,** 19–61.

ROBERTS, M. S., BALLINGER, L. N. & WEISS, M. (1999). Relative dispersions of intra-albumin transit times across perfused rat and elasmobranch livers and implications of intra- and inter species scaling of hepatic clearance using microsomal data. *J. Pharm. Pharmacol.* (in press).

ROBERTS, M. S., DONALDSON, J. D. & ROWLAND, M. (1988). Models of hepatic elimination: comparison of stochastic models to describe residence time distributions and to predict the influence of drug distribution, enzyme heterogeneity and systemic recycling on hepatic elimination. *J. Pharmacokin. Biopharm.* **16,** 41–83.

ROBERTS, M. S., FRASER, S., WAGNER, A. & MCLEOD, L. (1990a). Residence time distributions of solutes in the perfused rat liver using a dispersion model of hepatic elimination: 1. Effect of changes in perfusate flow and albumin concentration on sucrose and taurocholate. *J. Pharmocokin. Biopharm.* **18,** 209–234.

ROBERTS, M. S., FRASER, S., WAGNER, A. & MCLEOD, L. (1990b). Residence time distributions of solutes in the perfused rat liver using a dispersion model of hepatic elimination: 2. Effect of pharmacological agents, retrograde perfusions, and enzyme inhibition on Evans blue, sucrose, water, and taurocholate. *J. Pharmocokin. Biopharm.* **18,** 235–258.

ROBERTS, M. S. & ROWLAND, M. (1985). Hepatic elimination – dispersion model. *J. Pharm. Sci.* **74,** 585–587.

ROBERTS, M. S. & ROWLAND, M. (1986a). A dispersion model of hepatic elimination: 1. Formulation of the model and bolus considerations. *J. Pharmacokin. Biopharm.* **14,** 227–260.

ROBERTS, M. S. & ROWLAND, M. (1986b). A dispersion model of hepatic elimination: 2. Steady-state considerations. Influence of blood flow, protein binding and hepatocellular enzymatic activity. *J. Pharmacokin. Biopharm.* **14,** 261–288.

ROBERTS, M. S. & ROWLAND, M. (1986c). A dispersion model of hepatic elimination: 3. Application to metabolite formation and elimination kinetics. *J. Pharmacokin. Biopharm.* **14,** 289–307.

ROBERTS, M. S. & ROWLAND, M. (1986d). Correlation between *in-vitro* microsomal enzyme activity and whole organ hepatic elimination kinetics: analysis with a dispersion model. *J. Pharm. Pharmacol.* **38,** 177–181.

SCHOEMAKER, R. C., BURGGRAAF, J. & COHEN, A. F. (1998). Assessment of hepatic blood flow using continuous infusion of high clearance drugs. *Br. J. Clin. Pharmacol.* **45,** 463–469.

WEISS, M., BALLINGER, L. N. & ROBERTS, M. S. (1998). Kinetic analysis of vascular marker distribution in perfused rat livers after regeneration following partial hepatectomy. *J. Hepatol.* **29,** 476–481.

WEISS, M. & ROBERTS, M. S. (1996). Tissue distribution kinetics as a determinant of transit time dispersion of drugs in organs: application of a stochastic model for the rat hindlimb. *J. Pharmacokin. Biopharm.* **24,** 173–196.

YANO, Y., YAMAOKA, K., AOYAMA, Y. & TANAKA, H. (1989). Two-compartment dispersion model for analysis of organ perfusion system of drugs by fast inverse Laplace transform (FILT). *J. Pharmacokin. Biopharm.* **17,** 179–202.