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COMMENTARY

PHYSIOLOGICALLY BASED MODELS AND STRATEGIC EXPERIMENTS IN HEPATIC PHARMACOLOGY

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The central problem of modelling hepatic pharmacokinetics quantitatively is to transpose the relevant test-tube kinetics (known or postulated) into the setting of hepatocellular anatomy and microcirculation and, hence, to derive relations between quantities observable on the intact organ. In such a transposition, the mathematical form of the concentration-dependence of local metabolic rates (such as the Michaelis–Menten form) is commonly preserved, but a change in the numerical values of the kinetic constants (such as V_{max} and K_m) cannot be excluded *a priori* [1]. In general, a compromise between realism and parsimony is desirable: physiology must not be altogether lost in oversimplifications, but the interrelation between the model and data should not be trivialized by an excess of adjustable parameters [2]. As models of steady-state processes are particularly parsimonious, we defer discussion of time-dependent experiments to the penultimate section.

Substrates of liver enzymes are carried by blood through many (10^7–10^8) discontinuous capillaries (hepatic sinusoids) which are lined with hepatocytes containing the enzymes. Hepatic blood flow is manifolded through these capillaries from their common inlet and reunites in the hepatic vein. The activity of the enzymes depletes the substrates, so that concentration gradients develop along the flow, and these gradients in turn affect the global elimination rates of the substrates by the intact liver. Only at very high substrate concentrations is a flow-independent, biochemically determined elimination rate maintained by the saturation of the enzymes along all the capillaries. At the other extreme of low substrate concentrations and high enzyme densities, each sinusoid acts as a perfect sink of the substrate, so that global elimination is controlled by blood flow independently of biochemical parameters. The first task of any physiologically based modelling is to quantify elimination occurring between these two extremes.

A physiological approach: the undistributed perfusion model

We consider the steady hepatic elimination of a blood-borne substrate, due to irreversible metabolic conversion by hepatocellular enzymes, or to biliary excretion. Let the hepatic blood flow of rate F carry the substrate into the liver at the observed concentration c_i, and out of it at the observed concentration c_o. The steady rate of elimination, V, by the intact liver is then

$$V = F(c_i - c_o). \tag{1}$$

For any saturation kinetics, increasing c_i (and hence c_o) to sufficiently high values makes V tend to the maximum (saturated) value V_{max}, so that V_{max}/F is the maximum attainable arterial-venous concentration difference. Next, let Michaelis–Menten kinetics hold for the rate-determining step of the elimination process by each hepatocyte. Then, if all hepatocytes were presented with the same substrate concentration c, the hepatic elimination rate would be $V_{max}c/(c + K_m)$ with the half-saturating concentration K_m. In the intact organ, the substrate concentration presented to hepatocytes varies continuously from c_i at the inlet to c_o at the outlet, the concentration profile being itself the result of the interplay of local elimination with blood flow. What is the counterpart, for the intact organ, of the Michaelis–Menten relation in the test tube?

We consider a substrate rapidly equilibrated between blood and hepatocytes that are held in fixed positions in the hepatic blood flow by the scaffolding of the hepatic parenchyma, so that they provide a spatially distributed sink of the substrate. We put the x-axis along the blood flow, with inlet at $x = O$ and outlet at $x = L$, so that the steady concentration profile $c(x)$ satisfies $c(O) = c_i$, $c(L) = c_o$. The depletion of the substrate flux Fc by elimination in any interval x, $x + dx$ is expressed by applying equation 1 and Michaelis–Menten kinetics locally:

$$F\,dc = -[\rho(x)dx]c/(c + K_m). \tag{2}$$

Here $\rho(x)dx$ is the part of V_{max} in any interval x, $x + dx$, so that

$$\int_0^L \rho(x)dx = V_{max}. \tag{3}$$

Separating equation 2, integrating from O to L and using equation 3 gives [3]:

$$\frac{c_i - c_o}{K_m} + \ln\frac{c_i}{c_o} = \frac{V_{max}}{F K_m}. \tag{4}$$

This result may be interpreted as a generalization to saturation kinetics of the single-capillary model of first-order uptake in capillary physiology [4], initi-

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ated by Bohr [5] and exploited in hepatology by Brauer [6]. Let an organ comprise N capillaries (hepatic sinusoids) acting in parallel, in the functional rather than the anatomical sense. If all have the same values of c_i, K_m, maximum elimination capacity V_{max}/N and flow rate F/N, then the mixed venous concentration c_o is again given by equation 4 because its right-hand side can be written as $(V_{max}/N)/(F/N)K_m$, with N cancelling. The indifference of the resulting c_o to the manifolding of the blood flow through the parallel capillaries is characteristic of the undistributed (single capillary) model of elimination; it does not hold in the distributed model discussed below. We emphasize that the form of the function $\rho(x)$ does not affect equation 4: the enzyme distribution can vary arbitrarily along the blood flow and even amongst sinusoids, so long as equation 3 is satisfied. This important but often unrecognized [7, 8] result increases the realism and robustness of the undistributed perfusion model [3]: if spatial uniformity of enzyme distributions [$\rho(x) = \text{const} = V_{max}/L$] were a prerequisite, the model would be refuted simply by noting the observed zonal distributions of liver function along the hepatic blood flow [9].

If we define the logarithmic mean concentration

$$\hat{c} = \frac{c_i - c_o}{\ln(c_i/c_o)}, \quad c_o < \hat{c} < c_i \tag{5}$$

and eliminate F from equations 1 and 4, we find [3, 10]:

$$V = V_{max} \frac{\hat{c}}{\hat{c} + K_m}. \tag{6}$$

This formulation of the model has the same mathematical form as the test-tube Michaelis–Menten relationship, but with concentration given by equation 5 in terms of the physiological observables c_i, c_o. The elimination rate is the same as if all hepatocytes were presented with the same substrate concentration $\hat{c}$. While the Earth attracts bodies as if its mass were concentrated at its centre, no one believes, contrary to tangible evidence, that it is in fact so concentrated. Similarly, compartmental analysis should not be driven so far as to interpret equation 6 to mean that substrate actually has the uniform concentration $\hat{c}$ throughout the liver (with concentration jumps at the inlet and outlet), contrary to autoradiographic evidence of arterial-venous concentration gradients of a variety of substrates [11]. Indeed, equation 2 shows that the concentration gradient dc/dx is the very reason for $\hat{c}$ being the operative concentration in the context of Michaelis–Menten uptake.

Combining equations 1 and 4, we find

$$\frac{c_i}{c_o} = e^{\frac{V_{max} - V}{FK_m}} \tag{7}$$

for the inverse of the bioavailability c_o/c_i. The series expansion

$$\frac{c_i}{c_o} = 1 + \frac{V_{max} - V}{FK_m} + \tfrac{1}{2}\left[\frac{V_{max} - V}{FK_m}\right]^2 + \ldots \tag{8}$$

converges rapidly if $(V_{max} - V)/FK_m \ll 1$, either because $V_{max}/FK_m \ll 1$, or because V approaches V_{max} (saturation). If the series is broken off after the second (linear) term, and the resulting approximation is combined with equation 1, one obtains $V = V_{max}\, c_o/(c_o + K_m)$: the elimination rate is the same as if all hepatocytes were presented with the substrate concentration c_o. In this approximation to the perfusion model, one arrives at "venous equilibration" [12, 13] by the use of reasoning, without postulating arbitrarily that the venous concentration c_o is actually uniform throughout the liver (with a concentration jump from c_i to c_o at the inlet). This consideration delimits quantitatively the circumstances under which the venous equilibration model [12, 13] predicts approximately the same phenomena as the undistributed perfusion model [3], and under which it shares the rational basis of the perfusion model consistent with autoradiographic evidence [11].

When a series of pairs c_o, c_i is measured in an appropriately designed experiment, equations 1, 5 and 6 of the undistributed perfusion model permit the determination of the kinetic parameters V_{max} and K_m of the intact organ using, for example, the Lineweaver–Burk plot of test-tube biochemistry for the variables $1/V$, $1/\hat{c}$. This and other procedures have been used, with satisfactory results, to quantify the kinetic parameters of a variety of enzyme–substrate combinations in isolated perfused livers, intact animals and humans [3, 10, 14, 15]. The values of the kinetic parameters so determined presuppose the validity of the underlying model equations. It is therefore desirable to test parameter-free predictions of the model equations by specially designed experiments.

A set of model-testing experiments

We consider an isolated rat liver perfused in a recirculating system, eliminating steadily a substrate infused into the system at the steady rate $I = V$. The foregoing equations of the undistributed perfusion model then hold with I put in place of V. At some fixed $I < V_{max}$, let the flow rate F be changed from one steady value to another. Then $c_i - c_o = I/F$ (from equation 1) must change accordingly, but $\hat{c}$ should not change (because equation 6 does not involve F), provided that V_{max} and K_m remain unchanged. That is: if

$$\frac{\partial V_{max}}{\partial F} = 0 \quad \text{and} \quad \frac{\partial K_m}{\partial F} = 0, \tag{9}$$

then

$$\left(\frac{\partial \hat{c}}{\partial F}\right)_I = O, \tag{10}$$

where the suffix I indicates that the change in F is made at fixed I. The undistributed perfusion model predicts that, in this experimental design, the change in c_i and c_o with a change in F is such that $\hat{c}$, given by equation 5, is flow-independent. No adjustable parameters are involved in this prediction (a good model lives dangerously).

The validity of equations 9 for the intact liver does not follow from the biochemical meaning of V_{max} and K_m, because at sufficiently low flow rates sinusoids

collapse, depriving some hepatocytes of substrate, oxygen and ATP [16]. Then V_{max} as well as K_m may change with the flow rate. This is the familiar physiologic phenomenon of recruitment or de-recruitment of capillaries. To establish the validity of equations 9 within a range of flow rates high enough to avoid de-recruitment of sinusoids, it has been shown [17] that, under saturation with galactose, there is no statistically significant change in galactokinase V_{max} and oxygen consumption of a set of rat livers, until flow rates are reduced below $0.9\,ml \cdot min^{-1} \cdot (g\ liver)^{-1}$ on average. Furthermore, for elimination of alcohol by a set of rat livers, no statistically significant changes in V_{max} and K_m were found when these parameters were determined (from pairs of infusion rates, and equation 6) at two different flow rates above $0.9\,ml \cdot min^{-1} \cdot (g\ liver)^{-1}$ [18]. These results clear the way for interpreting unambiguously experimental tests of equation 10. These were done on sets of rat livers eliminating galactose [19] and propranolol [20]. Changes in $\hat{c}$ with F [kept above $0.9\,ml \cdot min^{-1} \cdot (g\ liver)^{-1}$] were not statistically significant, while changes in c_i and c_o were highly significant. The latter change demonstrated that, for interpreting these experiments, the expansion in equation 8 must be carried beyond the linear term: the "venous equilibration" approximation is inadequate.

The successful testing of equation 10 involves an interesting methodological trade-off. No numerical information about any kinetic parameters is obtained from the experiments; in exchange, confidence in the model is increased by its passing a particularly severe (parameter-free) experimental test. The null-prediction made by equation 10, reminiscent of the methodology of physics, is particularly suited to statistical evaluation (by slippage tests [18–20]).

Single-pass experiments and the distributed perfusion model

For single-pass (once-through) experiments with isolated perfused rat livers, the input concentration c_i is fixed, and changes in perfusate flow change the output concentration c_o as well as the elimination rate V. Experiments are often performed in the limit of first-order kinetics ($V \ll V_{max}$). Then equation 7 can be written as

$$\ln(c_i/c_o) = \frac{V_{max}}{F K_m}, \tag{11}$$

predicting that the plot of $\ln(c_i/c_o)$ against $1/F$ should be a straight line through the origin, with the slope V_{max}/K_m. As sinusoids collapse with decreasing flow rates (increasing $1/F$), V_{max} is reduced, the slope V_{max}/K_m falls, and $\ln(c_i/c_o)$ falls further below the straight line given by equation 11 at high flow rates. Brauer *et al.* [16] discussed such plots for the uptake of colloidal $CrPO_4$ by Kupffer cells of the rat liver and used the deviation of data from equation 11 to quantify the fraction of sinusoids open at each flow rate. Similar concave plots have since been obtained for other substrates, most recently for the elimination of taurocholate by rat liver [21]. However, the concave deviation from equation 11 is commonly found from data at flow rates at which de-recruitment does not occur [16, 17, 21]. The high statistical significance of these deviations ($P < 0.001$: [21, 22]) leaves little doubt that equation 11 and, hence, the undistributed perfusion model itself is at fault.

What is the explanation of these deviations from equation 11 at flow rates which are high enough to preserve the numbers of perfused sinusoids? If v_{max} and f, respectively, are the contributions by a single sinusoid to V_{max} and F of a whole liver comprising N sinusoids in parallel, then the undistributed model asserts that for all sinusoids $v_{max} = V_{max}/N$ and $f = F/N$. Such regularity cannot be expected in real capillary beds, as exemplified directly by quantitative studies of single capillaries [23]. We must expect statistical distributions of v_{max}, f and of v_{max}/f about their mean values over all sinusoids of a liver. Sinusoids with common c_i and K_m but different v_{max}/f will then have different output concentrations c_o; see equations 4 and 11 with V_{max}/F replaced by v_{max}/f. Then the mixed venous concentration $\bar{c}_o$ will be the flow-weighted mean of the c_o values from individual sinusoids, different from the c_o calculated from the undistributed model of a liver with the same organ values V_{max} and F, which we denote by $c_o(V_{max}/F)$. It is a general theorem [2] for steady saturable elimination by capillary beds that

$$\bar{c}_o \geq c_o(V_{max}/F) \tag{12}$$

holds for *any* dispersions of v_{max}, f and v_{max}/f over the set of parallel capillaries, with equality attained only at saturation: heterogeneity increases bioavailability. The distributed sinusoidal perfusion model [24–26] retains the equations of the undistributed model for the description of uptake by each sinusoid but quantifies effects of distributions of sinusoidal properties upon the relations between the quantities c_i, $\bar{c}_o$, F, and V observable on the organ.

Envisage the set of sinusoids of a given liver classified into groups labelled 1, 2 . . . n . . . , each consisting of sinusoids having the same v_{max}/f, a total maximum elimination capacity $V_{max}^{(n)}$ and a total flow rate $F^{(n)}$ (with $\Sigma\, V_{max}^{(n)} = V_{max}$ and $\Sigma\, F^{(n)} = F$). The groups may contain unequal numbers of sinusoids. A notional sub-organ made up of any one such group of sinusoids would be properly described by the undistributed sinusoidal perfusion model. The coefficient of variation, ε, of the distribution of v_{max}/f over all the notional sub-organs constituting the actual liver is given by [26]

$$\varepsilon^2 = \sum_n \frac{(V_{max}^{(n)}/V_{max})^2}{F^{(n)}/F} - 1. \tag{13}$$

ε^2 vanishes only when all sub-organs have the same values of $V_{max}^{(n)}/F^{(n)}$; it provides, in general, a measure of organ heterogeneity in the context of elimination. Equation 13 shows that ε^2 is left unchanged by any change in the organ flow rate F which is associated with proportionate changes in suborgan flow rates $F^{(n)}$ ($F^{(n)}/F = \text{const}$).

For a slightly heterogeneous liver ($\varepsilon^2 \ll 1$) eliminating substrate steadily by first-order kinetics in a single-pass system, equation 11 is now replaced by [25]

$$\ln(c_i/\bar{c}_o) = \frac{V_{max}}{F K_m} - \tfrac{1}{2}\varepsilon^2 \left[\frac{V_{max}}{F K_m}\right]^2, \tag{14}$$

neglecting higher-order terms in ε^2 estimated by a remainder term [25]. For any given V_{max}, F, c_i and ε, the $\bar{c}_o$ predicted by equation 14 is greater than the c_o predicted by equation 11: that is a particular realization of the general relation 12. As F is reduced ($1/F$ increased), the second term in equation 14 produces the concave deviation from the linear relation between $\ln(c_i/c_o)$ and $1/F$ predicted by equation 11. The distributed model thus accounts quantitatively [22, 25] for the aforementioned observations which are at variance with the undistributed model in the absence of de-recruitment, and in doing so it yields values of ε^2 characterizing the heterogeneity of the sinusoidal bed. While the values $\varepsilon^2 = 0.12$ [22] and 0.14 [25] were readily detected in this way, values of ε^2 below 0.18 could not have been detected (if present) in experiments with rat livers subjected to flow-changes in a re-circulating system [19, 27]. This interesting methodological difference extends the usefulness of the undistributed model in re-circulating systems such as the intact body.

Paradoxical effects of plasma albumins on elimination

What hindrance to elimination arises from the binding of a fraction of substrate to plasma proteins (especially albumins) in the perfusate? This question, fundamental for the delivery of some substrates and drugs to perfused organs, has another aspect. If the answer were known quantitatively, experimental variations in the bound fraction could be used to test the validity of models of elimination by intact organs [28, 29].

In an element of perfusate, the total concentration c of substrate consists of concentrations c_u of unbound and c_b of protein-bound substrate. For simplicity we consider total protein concentrations p such that the protein is so far from saturation by the substrate ($p \gg c_b$) that $p - c_b$ can be replaced with p. Then the difference between local rates of formation and of decomposition of the protein–substrate complex is

$$\sigma = k_{on}\, p\, c_u - k_{off}\, c_b, \qquad c_u + c_b = c \quad (15)$$

where k_{on} and k_{off} are the relevant absolute rate constants, determining the equilibrium dissociation constant $K_d = k_{off}/k_{on}$.

When the protein–substrate interaction is not perturbed by cellular uptake, as in large vessels or in a dialysis apparatus, we have $\sigma = 0$. Then equations 15 yield the equilibrium unbound fraction $c_u/c = f_{eq}$ of substrate:

$$f_{eq} = (1 + p/K_d)^{-1}, \quad (16)$$

which is independent of substrate concentration, and which can be varied at will by varying p [28, 29].

The traditional pharmacokinetic approach [12, 28, 29] makes two separate assumptions. First, it is assumed that only unbound substrate is involved in uptake leading to elimination (steady uptake of proteins is negligible in the present context). Second, it is assumed that when the accessible surface of a hepatocyte is presented with the total concentration c of substrate, then it is presented with the concentration $f_{eq}c$ of unbound substrate, with f_{eq} given by equation 16. As only unbound substrate enters the hepatocyte according to the first assumption, this second assumption ($\sigma = 0$ holding up to the hepatocyte surface) is incredible, especially when uptake is avid. If, nevertheless, it is adopted, the effect of protein-binding on steady elimination is simple. The left-hand side of equation 2 is unchanged, but on the right-hand side we must replace c by $f_{eq}c$ which is available for uptake: $f_{eq}c/(f_{eq}c + K_m) = c/(c + K_m/f_{eq})$. Consequently, throughout the foregoing model equations, K_m is to be replaced with K_m/f_{eq}. In particular, using equation 7 so modified to express the extraction fraction $E = 1 - c_o/c_i$, we obtain

$$E = 1 - e^{-f_{eq}\frac{V_{max} - V}{FK_m}}. \quad (17)$$

Here K_m is the Michaelis constant pertaining to unbound substrate: the enzyme is assumed not to interact with bound substrate in the hepatocyte, while unbound substrate is assumed to be equilibrated across the hepatocyte membrane. We will now focus on the difficulties of the traditional pharmacokinetic approach by formulating two characteristic paradoxes.

(A) *The low-extraction paradox.* At very low extractions, arterial-venous concentration differences are small, and all models of organ elimination become the same. This is reflected in retaining only the linear term in the expansion of the exponential in equation 17, with the result

$$E = f_{eq}\frac{V_{max} - I}{FK_m}, \quad (18)$$

where we set $V = I$ for steady uptake in a recirculating system. From equation 1 with $V = I$, we have the clearance $I/c_i = FE$ of the total substrate and the clearance $I/(f_{eq}c_i) = FE/f_{eq}$ of the unbound substrate (called "unbound clearance"). Note that our use of the concept of clearance is not confined to first-order kinetics. From equation 18 we see that the unbound clearance, $(V_{max} - I)/K_m$, is independent of f_{eq} and hence of the protein concentration p (equation 16). However, the calculated unbound clearance of prazosin by perfused rat liver is increased by some 69% by adding to the perfusate 14 g/l albumin, and is reduced by 51% by adding 0.85 g/l α-1-acid glycoprotein [30]. The unbound clearance of a variety of substrates by hepatocyte suspensions or monolayers is increased substantially by adding albumin to the perfusate (see Ref. 31). As the architecture of the liver is immaterial in all these situations, the low extraction paradox shows that this difficulty of the traditional pharmacokinetic view lies at the level of single hepatocytes interacting with perfusate.

(B) *The high-extraction paradox.* In order to consider high extraction fractions, we now turn to first-order kinetics ($V \ll V_{max}$). Writing $f_{eq}\, V_{max}/K_m = (CL)_{int}$ ("intrinsic clearance" of total substrate), equation 17 becomes

$$E = 1 - e^{-(CL)_{int}/F}. \quad (19)$$

Accordingly, $(CL)_{int}/f_{eq} = V_{max}/K_m$ is the unbound intrinsic clearance. For bromosulfophthalein (BSP) in a perfusate containing the physiological concentration 50 g/l albumin, Baker and Bradley [32] measured the very small unbound fraction, $f_{eq} =$

7.7×10^{-5}, and asked, therefore, how observed extraction fractions of BSP of 0.91 in humans and 0.50 in dogs are possible. To devise the highest possible value of $(CL)_{int}$, they assumed that the elimination rate was not limited by enzymatic elimination but by the diffusion of unbound BSP (with diffusion coefficient D_u) across the combined thickness δ of the perisinusoidal space and endothelial cells, into the accessible area A of hepatocytes (which sequestered all BSP on arrival):

$$(CL)_{int} = f_{eq}\, D_u A/\delta. \qquad (20)$$

With realistic values favourable to rapid uptake (in particular, $\delta = 10^{-4}$ cm), equation 20 yielded at most $E = 0.16$. Hence the earliest suggestion [32] that dissociation of the BSP–albumin complex is somehow facilitated by the hepatocyte surface: that is, that the use of f_{eq} in a perfusate compartment extending up to the hepatocyte surface is inappropriate in non-equilibrium conditions.

Non-compartmental approach to the albumin paradoxes

In avid uptake of unbound substrate, dissociation of substrate–protein complexes replenishes the depleted unbound substrate by mass-action. The rate of this replenishment varies continuously with the distance from the hepatocyte and cannot be calculated in terms of compartments. The key to understanding facilitation of the unbound clearance by albumin is in the identification of a new, albumin-dependent length [31] (denoted by $1/\lambda$ in what follows) which characterises the thickness of the non-equilibrium layer (within which $\sigma \neq 0$ in equation 15) adhering to the hepatocyte surface exposed to perfusate. That length is to be compared with the thickness δ of the familiar unstirred layer adhering to the hepatocyte surface, as for example in [32], within which all transport is by diffusion only. If x is the perpendicular distance from the hepatocyte surface, then the steady transport equations in the unstirred layer ($O \leqslant x \leqslant \delta$) are

$$-D_u \frac{d^2 c_u}{dx^2} = -\sigma, \; -D_b \frac{d^2 c_b}{dx^2} = \sigma \qquad (21)$$

where D_b is the diffusion coefficient of the substrate–albumin complex. The sink of c_u and source of c_b in equations 21 are σ given by equation 15. Multiplying the first of equations 21 by $k_{on}p/D_u$, the second by k_{off}/D_b and subtracting, we find

$$\frac{d^2\sigma}{dx^2} - \lambda^2 \sigma = 0, \qquad (22)$$

with

$$\lambda^2 = k_{on}p/D_u + k_{off}/D_b. \qquad (23)$$

The solutions of equation 22 are $\exp(\pm\lambda x)$ but as $\sigma = 0$ far from the hepatocyte, only the solution $\exp(-\lambda x)$ is of interest: $1/\lambda$ is the thickness of the non-equilibrium layer separating the hepatocyte from the equilibrated solution characterized by $\sigma = 0$. The thickness $1/\lambda$ falls as p is increased (equation 23). If $\delta < 1/\lambda$, stirring abolishes the non-equilibrium layer; but if $\delta > 1/\lambda$, it is diffusion across the non-equilibrium layer that limits avid uptake of the unbound substrate. Calculation shows that the intrinsic clearance of equation 20 must be replaced with [31]

$$(CL)_{int} = f_{eq}\, D_u A \frac{\lambda}{\tanh(\lambda\delta)}. \qquad (24)$$

For $\lambda\delta \ll 1$ ($\tanh(\lambda\delta) \simeq \lambda\delta$), equation 20 is recovered, but for $\lambda\delta > 2$ we find $\tanh(\lambda\delta) \simeq 1$ and equation 24 is reduced to $(CL)_{int} = f_{eq} D_u A/(1/\lambda)$: the fixed denominator δ in equation 20 is replaced by the smaller $1/\lambda$ which is reduced as p increases (equation 23). The unbound intrinsic clearance $(CL)_{int}/f_{eq}$ is no longer albumin-independent but increases with p as λ does. Using the best (though still provisional) values of BSP parameters appearing in equation 23 from published literature (see Ref. 31), one finds at $p = 50$ g/l of albumin [32] the estimate $1/\lambda = 0.38 \times 10^{-4}$ cm. Even if δ were only 10^{-4} cm, we have $\lambda\delta = 2.63$, $\tanh(\lambda\delta) \simeq 0.99$. The use in equation 19 of $(CL)_{int}$ from equation 24 (in place of $(CL)_{int}$ from equation 20) yields $E = 0.39$ (in place of 0.16). Considering the uncertainty of the parameter estimates, especially of k_{off} for BSP [31], this closer approach to observed values of E gives an encouraging illustration of the role of the non-equilibrium layer. More detailed quantitative applications of this concept have been made [31] to observed enhancements by albumin of hepatic clearances of unbound oleate, palmitate and BSP. However, the reduction of the hepatic clearance of unbound prazosin in rat liver by α-1-glycoprotein [30] cannot be explained by effects of the non-equilibrium layer at hepatocyte surfaces.

It is to be noted that, whereas albumin enhances the unbound intrinsic clearance of albumin-bound substrates, it reduces their total intrinsic clearance $(CL)_{int}$: as p is increased, the fall in f_{eq} outweighs the rise in $(CL)_{int}/f_{eq}$. Furthermore, the effects of the non-equilibrium layer are reduced, and the approximate validity of the traditional pharmacokinetic approach is restored, when the rate-determining step in elimination is the metabolism in the interior of the hepatocytes (rather than transport into hepatocytes). In the general case, both metabolism and transport influence the elimination rate. It is therefore inappropriate, in general, to test organ models of hepatic elimination by analysing observed effects of varying f_{eq} according to the traditional pharmacokinetic assumptions [28, 29, 31].

Interpretations of time-dependent elimination

Interpretations of elimination experiments with time-dependent concentrations require major extensions of the foregoing modelling. Consider an arterially injected bolus of substrate, fractionated by the vascular bed of the organ into elements which pass through the eliminating capillaries (sinusoids) and are re-united in the vein. There the mixed output concentration is seen with some time-dependence $\bar{c}_o(t)$, which is the flow-weighted mean of the outputs $c_o(t)$ from individual vascular pathways before mixing. The observed form of $\bar{c}_o(t)$, always more dispersed than the input $c_i(t)$, is due partly to the variety of transit times of the bolus fractions through the set of parallel vascular pathways and partly to

elimination along some part of each vascular pathway.

If a sinusoidal transit time during which elimination takes place (sometimes called contact time) is τ, then $v_{max}/f = \alpha\tau$, where $\alpha = v_{max}/(f\tau)$ is the maximum elimination rate per unit blood volume $f\tau$ of the sinusoid perfused at the rate f of flow. If the density of relevant hepatocellular enzymes were uniform along each sinusoid, and if it were the same for each sinusoid, then α would be the same constant along all sinusoids for a given substrate. If that is assumed, then the distribution of v_{max}/f over the sinusoids is the same as the distribution of τ. If the distribution of τ were known, the relation between the observed time–courses $c_i(t)$, $\bar{c}_o(t)$ could be interpreted quantitatively for each substrate in an organ represented by a set of sinusoids in parallel, each of which eliminates substrate according to the undistributed perfusion model.

Unfortunately, the distribution of τ is not observed. The instruments sampling c_i and $\bar{c}_o$ are inevitably placed well upstream and downstream of the sinusoids, with arterioles, venules and the other non-eliminating vascular domains included between them. When labelled inert indicators, suitably matched to the substrate [4], are used to determine the distributions of transit times T between the instruments, each T includes substantial times T' of passage through regions in which substrate is not eliminated. For any value of T, one must expect distributions of the times T' and τ (such that $T' + \tau = T$). Given an observed distribution of transit times T, elimination depends therefore also on the conditional probability density $\psi(\tau|T)$, defined as follows [33]: given a transit time T between sampling instruments, $\psi d\tau$ is the probability that T includes a time between τ and $\tau + d\tau$ spent in eliminating sinusoids. We note that, because of zones of metabolic activity in the liver [9], the same vascular pathway may have different values of τ for different substrates. In the simplest case of a bolus input eliminated (without back-diffusion) by first-order kinetics, the extraction fraction $E(T)$ at time T is given by [33]

$$E(T) = 1 - \int_0^T e^{-\alpha\tau}\psi(\tau|T)d\tau. \qquad (25)$$

This cannot be evaluated without models of $\psi(\tau|T)$, which fall into two broad classes: deterministic and probabilistic. Deterministic models assume that to each value of T there corresponds some unique value of τ. An influential example [34] of a deterministic model is obtained by assuming that $\psi = 0$ unless $\tau = a + bT$, where the positive constants a, b depend on the state of vasodilation or vasoconstriction of the vascular bed. In that case, equation 25 yields

$$E(T) = 1 - e^{-\alpha(a+bT)}. \qquad (26)$$

The simplest example [33] of the class of probabilistic models is to assume that, at any T, all values of τ between zero and T are equiprobable. This quantitative expression of our ignorance of the detailed situation yields, from equation 25,

$$E(T) = 1 - (1 - e^{-\alpha T})/\alpha T. \qquad (27)$$

The forms of $E(T)$ in equations 26 and 27 can describe various observed results of the multiple indicator diffusion (single injection) method [4], but existing experimental results cannot conversely determine the choice of $\psi(\tau|T)$, nor even give preference to either the deterministic or probabilistic class of models.

Whereas interpretations of elimination transients in real organs are burdened with the necessity to choose between hypothetical forms of $\psi(\tau|T)$, steady elimination is unaffected by the extrasinusoidal transit times T' and hence by the form of $\psi(\tau|T)$. This is because equations 4 and 14 are independent of the form of the distribution $\rho(x)$ of the eliminating enzyme (equations 2 and 3): we can set $\rho(x) = 0$ along any non-eliminating part of any vascular pathway without effect on the relations between the steady observable concentrations c_i and c_o or $\bar{c}_o$. We note here another methodological trade-off: steady-state experiments yield less information than transients (for example, on enzyme distributions along the blood flow) but require fewer speculative assumptions.

We have assumed so far that transport of indicators and substrates along the blood flow in capillaries and sinusoids is predominantly convective [2–4, 23, 33, 34]: bolus fractions do not disperse appreciably while travelling through a capillary, so that the dispersion of $\bar{c}_o(t)$ seen in the vein is due to distributions of transit times of the bolus fractions. This picture is supported directly by work with single capillaries [2, 23]. In the intact liver it has been shown [35] that, after plausible corrections for volumes of distribution, a variety of substances injected arterially in the same bolus have the same venous outflows $\bar{c}_o(t)$ as labelled red cells, despite having diffusion coefficients differing by many orders of magnitude. For these reasons the modelling of substrate diffusion along blood flow in capillary beds [36] has not been influential: "the intravascular pathways traversed by tracer molecules vary enough to give practically all the dispersion (of $\bar{c}_o(t)$) observed" [37].

Interest in diffusion-like dispersion of substrates along the flow through hepatic sinusoidal beds has been revived recently by Roberts and Rowland [38]. They envisage the molecular diffusion coefficient [36, 37] replaced by a much larger dispersion coefficient representing effects of microscopic convective eddies related to intrahepatic interconnections between sinusoids. This dispersion coefficient is primarily a property of the anatomy of the intact sinusoidal bed and of the dynamics of its perfusion and depends only slightly on the molecular diffusion coefficients of indicators and substrates. In particular, the dispersion coefficient must be expected to depend on the total hepatic blood flow in some essential but hitherto unspecified way [38]. Current attempts to model this flow-dependence [22] are confined to limiting situations which do not cover the range of the strategic flow-change experiments discussed in the first part of this commentary. Interpretations of these experiments in terms of the revived dispersion model [38] must, therefore, await its completion.

Conclusion

Hepatic physiology and clinical pharmacokinetics

have now both developed so far that the traditional gap between these disciplines should be bridged. This requires that pharmacokinetic models should be physiologically reasonable and, on the other hand, that physiologic models should be clinically useful. An important connection between the two disciplines is that both must give an account of effects of hepatic blood flow, and of effects of protein binding, on phenomena central to them. It is an essential part of such a program of unification that a more quantitative approach should be attempted than has been customary hitherto [8]. The present commentary emphasizes this point of view.

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