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Dissociation from albumin: A potentially rate-limiting step in the clearance of substances by the liver

(drug binding/organic anions/bilirubin/transport/models)

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ABSTRACT **The hepatic uptake rate for certain albumin-bound drugs and metabolites correlates poorly with their equilibrium unbound concentration in the plasma, suggesting that binding equilibrium may not always exist within the hepatic sinusoids. Currently available models for the uptake process assume binding equilibrium and, thus, cannot be used to investigate this possibility. This report presents a more general model that treats plasma-bound and free concentrations separately. A solution is provided that specifies the hepatic uptake rate as a function of the total plasma concentrations of the transported substance and of binding protein and the rate constants for influx, efflux, elimination, association, dissociation, and flow. Analysis of this solution indicates that hepatic uptake may be limited by the rate of plasma flow, dissociation from the binding protein, influx into the liver, cellular elimination, or any combination of these processes. The affinity and concentration of the binding protein strongly influence which of these steps are rate-limiting in any given case, and binding equilibrium exists within the hepatic sinusoids only for binding protein concentrations greater than a specified value (the ratio of the uptake and association rate constants). The precise conditions under which each step is rate-limiting and the kinetic behavior expected when two or more steps mutually limit uptake are provided. The results are compatible with previously reported data for the uptake of certain albumin-bound ligands such as bilirubin, and they offer an alternative to attributing these kinetics to the presence of an albumin receptor.**

The degree to which a drug or metabolite is bound to albumin in plasma is recognized as an important determinant of its rate of removal by the liver and other tissues. Indeed, it has long been assumed that only the unbound form is available for uptake (1). This assumption is not necessarily valid, however, because the bound substance always retains some free energy (2), which makes it a potential substrate for an uptake process. Recent reports have, in fact, suggested that for several albumin-bound substances (subsequently referred to as ligands), the rate of hepatic removal correlates much more closely with the bound than with the equilibrium-free concentration (3, 4). To account for these and related data, it has been proposed that uptake may occur not only from the free ligand pool, but also (less efficiently) from the bound ligand pool by a mechanism involving transient binding of albumin to receptor sites on the cell surface (5, 6). According to this model, the latter pathway predominates for tightly bound ligands such as bilirubin because of their very small unbound concentrations in plasma.

Although the albumin-receptor model is attractive, it is important to consider whether the traditional view that uptake depends only on the unbound ligand concentration at the liver cell surface might not also accommodate these observations. Most traditional models assume that equilibrium exists between bound and free ligand within the hepatic sinusoids. Recent evidence, however, suggests that the rate of dissociation of some ligands from albumin may be insufficient to maintain equilibrium when hepatic uptake is rapid (3, 6). To address these important issues, a new formulation of the traditional uptake model has been developed that treats bound and free ligand as separate plasma compartments. The resulting analysis indicates that the kinetic behavior that has previously been attributed to an albumin receptor can also be produced by the traditional uptake model. In addition, this approach provides numerous insights into the kinetic behavior expected for the traditional model, which should prove useful in interpreting the results of pharmacokinetic and transport studies.

METHODS

For this analysis, the liver is treated as a collection of equivalent tubular sinusoids, each of which is divided into N axial elements (see Fig. 1). Within each element plasma-bound and free ligand are treated as separate compartments that exchange according to the rate constants for complex dissociation (r_2) and formation ($r_1 \cdot A$). The concentrations of free ligand (L), albumin-ligand complex (C), and free albumin (A) entering the first element are the equilibrium values of the bulk plasma, while each subsequent element receives the effluent of the element which preceeds it. The net rate of uptake (or removal) for a single element is found by solution of the steady-state mass balance equations (see *Appendix*). The rate for the entire sinusoid is found by summation of the rates for all elements. When implemented on a small computer, this approach allows convenient plotting of the predicted net uptake velocity as a function of any one or more of the model parameters.

The conditions under which each step in the uptake process (i.e., plasma flow, dissociation, influx, and elimination) is rate limiting were determined by inference from the general solution. The equation that governs uptake when each step is rate-limiting was first determined, and then the conditions under which the general solution simplifies to that equation were found (see *Appendix*). The resulting conditions are those for which that step is solely responsible for determining the uptake rate.

THEORY

This is a linear model (i.e., transport and elimination are assumed not to be saturable for the range of ligand concentrations used). Saturation of the albumin-binding sites at higher concentrations of ligand is, however, permitted. This model extends existing models to cover conditions for which binding equilibrium may not be present. When a single sinusoidal element is used ($N = 1$), the model is structurally similar to the "well-stirred" or "venous equilibrium" model, while for large values of N it is similar to the "parallel tube" or "sinusoidal" model (7). The conditions under which each step is rate-limiting were derived for $N = 1$ and thus, strictly speak-

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ing, apply only to the well-stirred model. However, the number of elements used had little effect on the resulting velocity except when the single-pass extraction of ligand exceeded 80%–90%. Ten elements were arbitrarily used for all graphic simulations in this study, although results in each case were effectively independent of the number of elements for $N > 3$. This same basic approach has been used previously to investigate the effects of slow rates of dissociation, although with a different emphasis (8).

The solution for a single sinusoidal element at steady state is given by Eq. 1, where V is the net hepatic uptake (removal) velocity (see units below), $L_{t,in}$ is the total entering ligand concentration, A_t is the total entering albumin concentration (assuming one binding site per albumin molecule), L_{in} is the entering free ligand concentration, and S is a composite rate constant that incorporates the rate constants for influx (k_1), efflux (k_2), and elimination (k_3), and that governs the rate of removal of free ligand from the sinusoid. Q, r_1, and r_2 are the rate constants for flow, association, and dissociation. To simplify subsequent analysis by allowing direct comparison between rate constants, V is expressed per unit sinusoidal volume ($mol \cdot sec^{-1} \cdot cm^{-3}$), while Q is expressed as the rate at which plasma within the element is replaced by flow (sec^{-1}). r_1 has units of $sec^{-1} \cdot M^{-1}$; all other rate constants have units of sec^{-1}.

$$V = \frac{r_1(L_{t,in} - A_t) - (r_2 + Q)(1 + S/Q) + \sqrt{[r_1(L_{t,in} - A_t) - (r_2 + Q)(1 + S/Q)]^2 + 4r_1(1 + S/Q)(r_2 \cdot L_{t,in} + Q \cdot L_{in})}}{2r_1(1/Q + 1/S)} \quad [1]$$

Where

$$S = \frac{k_1 \cdot k_3}{k_2 + k_3} \quad [2]$$

From Fig. 1, it is evident that ligand in the bulk plasma must pass through several intermediate compartments before it can be eliminated by the liver. A small number of parameters govern exchanges between these compartments, and their relative values determine which step or steps in uptake will be rate-limiting in any given case. In addition to those previously listed, these include $r_1 \cdot A$, which governs the rate of association of ligand to albumin (sec^{-1}), and $S[K_d/(A + K_d)]$ (S times the equilibrium free ligand fraction, where K_d is the equilibrium dissociation constant r_2/r_1), which governs the rate of hepatic removal of total ligand from the sinusoid under equilibrium conditions (sec^{-1}).

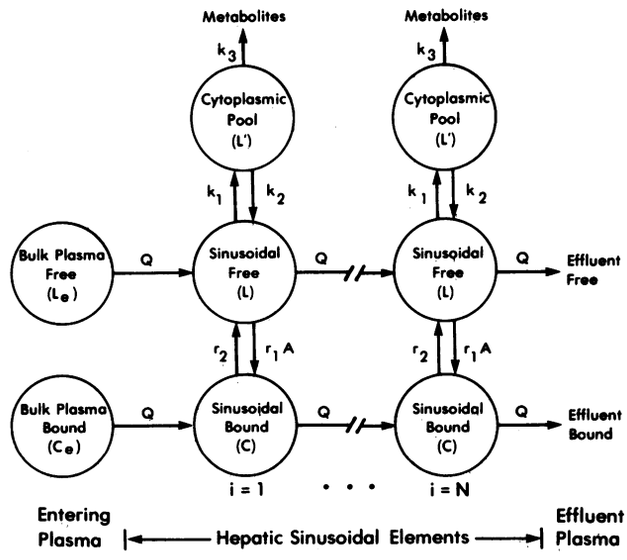

FIG. 1. Model in which the liver is represented by a single tubular sinusoid divided into a series of elements.

RESULTS

Four discrete steps may limit the net rate of ligand uptake at steady state: plasma flow, dissociation from albumin, influx, and elimination. The conditions under which each of these steps is rate-limiting are given in Table 1.

Flow-Limited Uptake. Uptake is exclusively flow-limited whenever the uptake rate is equal to the product of the flow rate and the total ligand concentration. For the general solution to simplify to this equation, the rate constant for flow must be much smaller than both the rate constants for total ligand uptake and for dissociation (Table 1). These conditions assure that neither uptake nor dissociation can be rate-limiting.

Dissociation-Limited Uptake. Uptake is limited by the rate of dissociation from albumin whenever the uptake rate is equal to the product of the dissociation rate constant and the bound ligand concentration. The first condition requires that flow must be large enough to avoid flow-limited uptake, but small enough that dissociation remains the primary source of free ligand within the sinusoid. The second condition specifies that the albumin concentration must be large enough that most ligand is bound, but small enough that binding equilibrium is not present within the sinusoid (see below).

Intrinsically Limited Uptake. Uptake is limited by the intrinsic capacity of the liver to remove ligand from the sinusoid whenever the uptake rate is equal to the product of the intrinsic uptake rate constant (S) and the equilibrium free ligand concentration (L_e). Two cases exist. When k_2 is $<<k_3$, S simplifies to k_1 and uptake is influx-limited. In contrast, when k_3 is $<<k_2$, equilibrium is present between the

Table 1. Conditions for which uptake is limited by a single step

Limiting step	Characteristic behavior	Applicable conditions
Flow	$V = Q \cdot L_{t,in}$	$Q << S\frac{K_d}{A + K_d}$ and $Q << r_2$
Dissociation	$V = r_2 \cdot C_{in}$	$S\frac{K_d}{A + K_d} << Q << S$ and $r_2 << r_1 \cdot A << S$
Influx	$V = k_1 \cdot L_e$	$S\frac{K_d}{A + K_d} << Q$ and $S << r_1 \cdot A$ and $k_2 << k_3$
Elimination	$V = \frac{k_1}{k_2} k_3 \cdot L_e$	$S\frac{K_d}{A + K_d} << Q$ and $S << r_1 \cdot A$ and $k_3 << k_2$

In each case, conditions shown are sufficient to reduce the general solution (Eq. 1) to the simplified equation shown. All condition terms have units of sec^{-1}. V is net uptake velocity per unit sinusoidal volume at steady state, A is the concentration of unoccupied protein binding sites, L is the equilibrium free ligand concentration, C_{in} is the equilibrium bound ligand concentration, Q is the rate of plasma exchange within the sinusoid due to flow, and r_1, r_2, k_1, k_2, and k_3 are the rate constants for binding, dissociation, influx, efflux, and elimination. K_d is the equilibrium dissociation constant of binding sites (r_2/r_1) and S is a composite rate constant $[k_1 \cdot k_3/(k_2 + k_3)]$ that reflects the intrinsic uptake capacity of the liver at steady state.

plasma and cytoplasm, and S simplifies to the product of the equilibrium partition coefficient (k_1/k_2) and the elimination constant (k_3). Uptake in this case is elimination-limited. For uptake to be intrinsically limited, the rate constant for flow must be greater than that for removal of total ligand by the liver (avoiding flow-limited uptake) and the rate of uptake by the liver must be a small fraction of the rates of binding and dissociation (avoiding dissociation-limited uptake).

Intermediate Cases. Each of these special cases applies only to a limited range of non-overlapping conditions. In practice, experimental conditions rarely fall neatly into any one of these categories, and more than one step will be involved in determining the uptake rate. For example, whenever the single-pass extraction of ligand is measurably large, plasma flow is at least partially rate-limiting to uptake. Likewise, whenever the rate of efflux from the liver at steady state is a significant fraction of influx, elimination is at least partially rate-limiting to uptake.

Since the free albumin concentration (A) and the affinity of the albumin binding sites (K_d) appear in many of the conditions in Table 1, these parameters may greatly influence which step limits uptake. Several cases are possible.

[$S << Q$]. The simplest situation occurs when the rate constant for intrinsic hepatic removal is much smaller than the rate constant for flow. In this case, the rate at which free ligand flows into the sinusoid is always much greater than the rate of removal by the liver, and uptake is intrinsically limited for all values of A and K_d. Fig. 2 (Case A) demonstrates the effect of varying the degree of albumin binding for typical parameter values. Binding is expressed by the ratio A/K_d (the ratio of the free albumin concentration to the equilibrium dissociation constant), which is equivalent to the ratio of bound to free ligand in the entering plasma. Physiologic values of A/K_d vary from 0 (for unbound substances) to $\approx 10^4$ (for tightly bound ligands such as bilirubin).

[$Q << S << r_2$]. When Q is $<S$, and S is $<r_2$, uptake will be flow-limited for lower values of A/K_d and intrinsically limited for higher values. This transition occurs because increasing the amount of binding reduces the rate of hepatic removal without decreasing the rate at which ligand enters the sinusoid. Fig. 2 (Case B) shows the effect of making $S > Q$, with other parameters unchanged. Uptake remains flow-limited even for A/K_d values somewhat >1. For this to be possible, the rate of dissociation must be rapid compared to the rate of removal.

[$Q << S$ and $r_2 << S$]. For slower rates of dissociation, dissociation-limited uptake becomes possible. Fig. 2 (Case C) shows the effect of reducing r_2 until it is <S with other parameters unchanged. Uptake remains flow-limited at the lowest binding values and intrinsically limited at the highest. However, a region now exists between these extremes for which uptake is dissociation-limited. Here, the uptake process removes free ligand so efficiently that dissociation cannot keep up. Uptake within this region is proportional to the bound ligand concentration (since this determines the rate of dissociation) and independent of the equilibrium free ligand concentration (since binding equilibrium is not present at the site of uptake).

The lower limit of the dissociation-limited region is $A/K_d > 1$, which is equivalent to

$$A > r_2/r_1. \quad [3]$$

The upper limit of this region is the free albumin concentration necessary to produce binding equilibrium within the sinusoid. Higher values of A favor equilibrium because they reduce the removal rate (by reducing the free ligand concentration) while not affecting the rate of dissociation. Since equilibrium requires that the rates of ligand binding and dissociation be effectively equal, the rate at which free ligand is cleared by the liver ($S \cdot L$) must be small with respect to the rate of association ($r_1 \cdot A \cdot L$)

$$S \cdot L << r_1 \cdot A \cdot L. \quad [4]$$

Solution for A gives the albumin concentration necessary to produce sinusoidal binding equilibrium:

$$A >> S/r_1. \quad [5]$$

Since A must be below this value for uptake to be dissociation-limited, the range of free albumin concentrations over which uptake is limited by dissociation is given by:

$$r_2/r_1 < A < S/r_1. \quad [6]$$

Since r_1 is a common divisor in both the lower and upper limits, it follows that a necessary condition for such a range to exist is

$$r_2 < S. \quad [7]$$

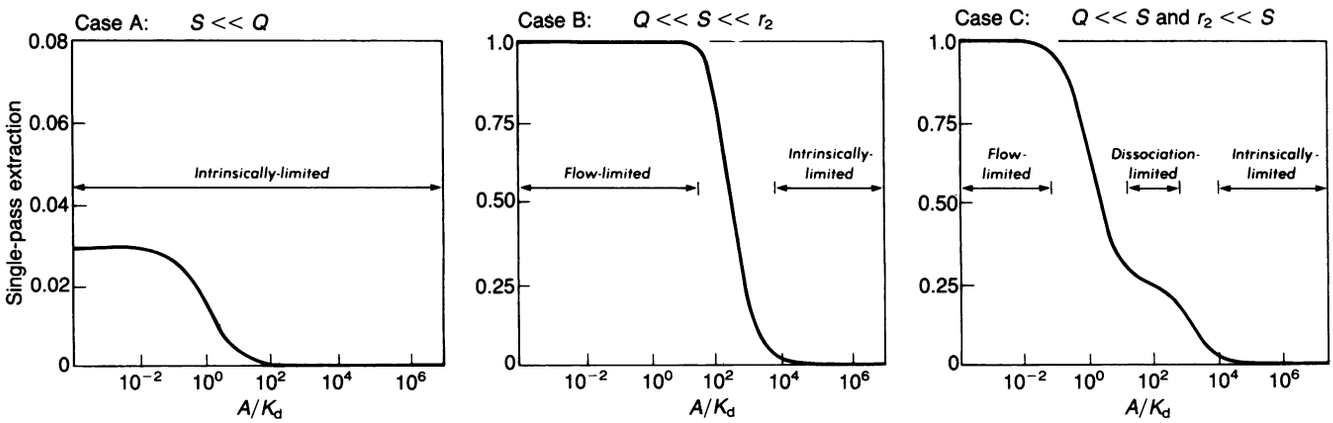

FIG. 2. Effect of the degree of albumin binding (A/K_d) on the fraction of ligand that is extracted by the liver in a single pass. Case A [$S << Q$], uptake is limited by intrinsic uptake capacity of liver for all binding levels. Case B [$Q << S << r_2$], uptake is flow-limited for lower levels and intrinsically limited for higher levels. Case C [$Q << S$ and $r_2 << S$] is similar to case B except that dissociation-limited uptake may occur at intermediate levels. In these simulations, A was varied and K_d was held constant. Parameter values are as follows: Case A: $S = 0.01$ sec^{-1}, $Q = 0.33/N$ sec^{-1}, $r_2 = 1000$ sec^{-1}, $r_1 = 10^{10}$ sec$^{-1} \cdot$M^{-1}, $L_{t,in} = 10^{-11}$ M, and $N = 10$. Case B: same as case A except that S has been increased to 100 sec^{-1}. Case C: same as case B except that both r_1 and r_2 have been divided by 10^4.

These conditions are equivalent to those found previously by inference from the general solution (Table 1).

Effect of Varying Ligand at Constant Albumin. Simulations based on Eq. 1 indicate a linear response between the total ligand concentration and the uptake rate, providing only that the binding capacity of the albumin is not significantly saturated (data not shown). Uptake is proportional to the total ligand concentration for all values of all parameters, regardless of which uptake step is limiting.

Effect of Varying Both Ligand and Albumin. For $A > K_d$, the equilibrium free ligand concentration depends only on the molar ratio of L to A, and not on their absolute concentrations. Thus, simultaneous variation of A_t and $L_{t,in}$ at a fixed molar ratio will always cause the uptake velocity to approach a limiting value ($S \cdot L_e$). For values of S too small to produce flow- or dissociation-limited uptake, the limiting uptake rate is reached for $A >> K_d$ (Fig. 3, Case A). When S is larger, the albumin concentration required to produce intrinsically limited uptake will be greater. The saturation-like behavior that results is due to the transition from flow-limited to intrinsically limited uptake (Case B) or from dissociation-limited to intrinsically limited uptake (Case C) and is not produced by actual saturation of a receptor site or transport mechanism.

DISCUSSION

The current study indicates that at least four different steps may limit the rate of hepatic uptake of protein-bound drugs and metabolites. Emphasis has been placed on the role of protein binding in determining the rate-limiting step, since failure to fully consider this factor is a potential source of confusion within the literature. In particular, studies that involve variation of the albumin concentration may be difficult to interpret if the rate-limiting uptake step is changed.

Although sinusoidal bound and free ligand are treated as separate compartments in this analysis, in many cases this is unnecessary. However, the current study indicates that the sinusoidal plasma will behave as a single compartment only when the free binding protein concentration is $>S/r_1$. Since S and r_1 vary among ligands, two classes of ligands must exist. For the first "equilibrative" class, the physiologic concentration of albumin or other binding protein is $>S/r_1$ (due to low values of S, large values of r_1, or both). For this class, the plasma within the hepatic sinusoids will behave as a single compartment and the uptake rate at a steady state will reflect the equilibrium free concentration of the ligand. Warfarin appears to belong to this class (9). The second, "non-equilibrative" class includes those ligands for which physiologic binding protein concentrations are insufficient to maintain binding equilibrium within the sinusoids. For these compounds, the sinusoidal plasma will behave as separate bound and free compartments. Since dissociation is rate-limiting, uptake will reflect the bound rather than the equilibrium free concentration. This type of behavior has been observed in the perfused rat liver for bilirubin, sulfobromophthalein, long chain fatty acids, taurocholate, and rose bengal (3–6, 10–12). However, the albumin concentrations used in these studies were typically lower than physiologic, and it remains to be determined if dissociation of any of these ligands is rate-limiting to uptake under physiologic conditions.

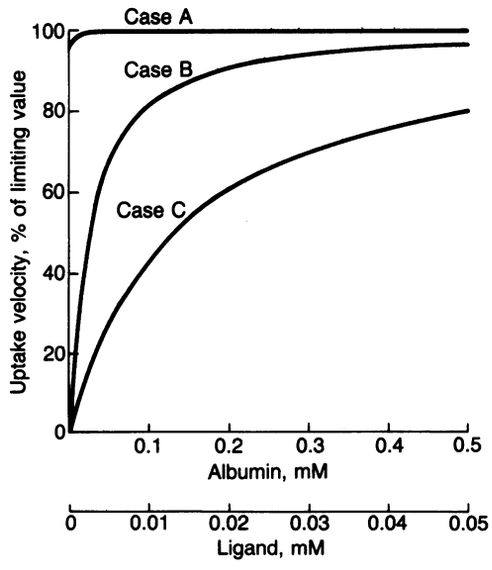

FIG. 3. Effect of varying both ligand and albumin concentrations at a fixed molar ratio (0.1). Uptake approaches a limiting velocity in each case as albumin concentration becomes large enough to produce intrinsically limited uptake. Parameter values are the same as for Fig. 2. Limiting velocities are as follows: Case A, 0.11 pmol·sec^{-1}·cm^{-3}; Cases B and C, 1.1 nmol·sec^{-1}·cm^{-3}.

Available experimental data are frequently insufficient to determine which step in uptake is rate-limiting. Although it is usually possible to determine if flow is limiting from the measured or calculated single-pass extraction of ligand, it is more difficult to discriminate among the other uptake steps, because the rate constants governing these processes are rarely known. The data are most difficult to interpret when they have been obtained at a single binding protein concentration, since a linear relationship between net uptake velocity and total ligand concentration is expected regardless of which steps are limiting. Even if saturation of a membrane carrier or elimination process can be demonstrated at higher ligand concentrations (indicative of intrinsically limited uptake), it cannot be assumed that this same process remains rate-limiting at lower concentrations of ligand, which are often more physiologic. Saturation necessarily lowers the effective value of S and may, thus, artifactually convert flow- or dissociation-limited uptake to intrinsically limited uptake.

Several considerations limit the predictive value of this analysis. First, for simplicity, albumin has been assigned a single class of non-interacting binding sites despite the fact that it often displays multiple sites of variable affinity. Although an exact solution for the behavior of the traditional model under these more general conditions is not available, numerical simulations suggest that the effect is to broaden the range of albumin concentrations over which transitions between different uptake steps occur (unpublished data).

The second major limitation is that values for several of the parameters necessary to solve Eq. 1 are poorly known. For a variety of ligands, including bilirubin, oleate, taurocholate, and sulfobromophthalein, the single-pass extraction by the perfused rat liver in the absence of albumin is 0.95–0.998 (unpublished data), suggesting that S must be at least 1–2 sec^{-1}. If, however, the sinusoids are of unequal length or are perfused unequally, S could be much larger. Estimated values for r_2 for several of these ligands range from 0.003 to 0.04 sec^{-1} (13–16). Since these values are well below the minimum estimate for S, Inequality 7 is satisfied and a range of albumin concentrations must exist for these ligands for which uptake is dissociation-limited.

Estimates for r_1 are less certain. Early events in association appear to be diffusion-limited, with rate constants for several tightly bound ligands ranging from 1–40 $\times$ 10^6 M^{-1}·sec^{-1} (13, 17–22). However, the rate-limiting step in complex formation may not be this initial interaction, but rather the much slower process of conformational relaxation of the albumin molecule (typically on the order of 2 sec^{-1}), which is believed to increase the affinity of the interaction

(13, 17–21). Because of this large uncertainty in the value of r_1, it is not possible to determine from inequality **5** whether physiologic albumin concentrations are sufficient to maintain sinusoidal binding equilibrium for these ligands.

"Saturable" uptake kinetics similar to those shown in Fig. 3 have recently been found for long chain fatty acids (3), bile acids (4), bilirubin (11), sulfobromophthalein (10, 11), and rose bengal (12). It has been proposed that these kinetics reflect the presence of sites on the liver cell surface that transiently bind albumin and catalyze the transfer of ligands from albumin to the cell. Such a mechanism has been independently supported by the demonstration of saturable binding of albumin to liver cells, and it can be shown that some form of catalyzed dissociation must exist to explain the observed rates of uptake if the published dissociation rate constants are correct (5, 6). Nevertheless, the traditional model presented here remains a viable alternative to the albumin-receptor model. Careful measurement of the rate constants for dissociation of these ligands from albumin under physiologic conditions appears to be the most promising method for discriminating between these alternative uptake models.

APPENDIX

Derivation of Net Uptake Rate for a Single Sinusoidal Element. At steady state, the values for L, C, and L' in each element are constant:

$$\dot{L} = Q(L_{in} - L) - k_1 \cdot L + k_2 \cdot \beta \cdot L' - r_1 \cdot A \cdot L + r_2 \cdot C = 0 \quad [A1]$$

$$\dot{C} = Q(C_{in} - C) + r_1 \cdot A \cdot L - r_2 \cdot C = 0 \quad [A2]$$

$$\dot{L}' = k_1 \cdot L - (k_2 + k_3) \cdot \beta \cdot L' = 0 \quad [A3]$$

where L_{in} and C_{in} are the concentrations of free ligand and complex flowing into the element, and β is the ratio between the cytosolic and plasma volumes. Eq. **A3** is solved for L' and the result is substituted in Eq. **A1**, which is solved for C and substituted in Eq. **A2**. After substitution of $A_t - C$ for A and $L_{t,in}$ for $L_{in} + C_{in}$, the result is a quadratic equation in L.

$$r_1\left(1 + \frac{S}{Q}\right)L^2 - \left[r_1(L_{t,in} - A_t) - (r_2 + Q)\left(1 + \frac{S}{Q}\right)\right]L - r_2 \cdot L_{t,in} - Q \cdot L_{in} = 0 \quad [A4]$$

where S is a composite rate constant equal to $k_1 \cdot k_3/(k_2 + k_3)$. Solution of this equation by the quadratic formula gives L as a function of A_t, $L_{t,in}$, L_{in}, and the relevant rate constants (only the positive root gives valid results). Multiplying by S yields the expression for the net uptake velocity at steady state (V), and it is given as Eq. **1** in the text.

Special Cases. Each step in the uptake process is characterized by a simple equation (Table 1) that represents a special case of the general solution. The conditions under which the general solution simplifies to each special case were found as follows.

Since the general solution was found by the quadratic formula, it has the form

$$V = \frac{-b + \sqrt{b^2 - 4 \cdot a \cdot c}}{2 \cdot a}, \quad [A5]$$

while the equation for each special case has the form:

$$V = d \quad [A6]$$

where a, b, c, and d are expressions in A_t, $L_{t,in}$, L_{in}, r_1, r_2, k_1, k_2, k_3, and Q. The conditions for simplification were found by determining the conditions under which these equations are approximately equal. Squaring the resulting expression and rearranging gives the general condition for simplification

$$4 \cdot a^2 \cdot d^2 + 4 \cdot a \cdot b \cdot d + 4 \cdot a \cdot c + b^2 \simeq b^2. \quad [A7]$$

The specific conditions were found in each case by expansion of Eq. **A7** and finding the conditions necessary to eliminate the appropriate terms. In some cases, the resulting conditions were combined to give the conditions listed in Table 1. For example, the second condition for dissociation-limited uptake combines the simple conditions, $A << S/r_1$, $A >> K_d$ and $r_2 << S$, each of which was useful in eliminating terms.

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