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# PLASMA BINDING AND HEPATIC DRUG ELIMINATION

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The reversible binding of endogenous compounds, drugs, and other xenobiotics to plasma proteins has been of pharmacological interest and importance for many years. A large body of knowledge has, therefore, been developed concerning the measurement of binding, interpretation of binding isotherms, and various factors affecting the phenomenon both in vitro and in vivo. A number of reviews of these areas describe such information and provide a broad foundation to the theoretical and practical aspects of drug binding (Anton and Solomon 1973; Jusko and Gretch 1976; Kragh-Hansen 1981; Wilkinson 1983). However, it is only recently that a clearer understanding has developed of the quantitative consequences of such binding in the in vivo disposition of drugs, particularly their elimination by the liver.

Traditionally, intravascular binding has been conceived to have a restrictive effect upon drug elimination. This is because only the unbound drug is able to penetrate biological membranes and organ uptake is limited to this moiety. Accordingly, it would be expected that the extraction of very highly bound (> 98 percent) drugs would be quite small and elimination slow. However, experimental studies indicate that this is not necessarily the case; some drugs are very efficiently eliminated despite their extensive plasma binding. This chapter will describe the current status and putative mechanisms for such observations with regard to the liver, as well as the consequences of plasma binding on the drug concentration/time profile in the blood.

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## PERFUSION-LIMITED HEPATIC ELIMINATION

A unifying and quantitative relationship between vascular binding and an organ's drug eliminating ability was initially established with the so-called "well-stirred" or "venous equilibration" model (Wilkinson and Shand 1975; Pang and Rowland 1977). This related organ clearance of total drug ($CL_{tot}$) to three physiological determinants (Equation 1); the parenthetic term being equivalent to the organ's extraction ratio (E).

$$CL_{tot} = QE = Q\left[\frac{f_B^u CL_{int}^u}{Q + f_B^u CL_{int}^u}\right] \qquad \text{Equation 1}$$

where Q is the blood flow through the organ; $CL_{int}^u$ is the ability of the elimination process to irreversibly remove drug from tissue water, termed free intrinsic clearance; and $f_B^u$ is the fraction of drug unbound in the blood. This latter determinant is related to the more conventionally determined unbound drug in the plasma ($f_P^u$) by the blood:plasma concentration ratio, which itself may be dependent on plasma binding ($f_B^u = f_P^u(1 - H + HKpf_P^u)$), where H is the hematocrit and Kp is the partition ratio of drug concentration in the formed elements of the blood to the unbound level in the plasma (Tozer 1981). Accordingly, clearance is independent of tissue binding and, therefore, volume of distribution, and the effect of plasma binding depends on the relative magnitude of free intrinsic clearance to organ blood flow. When $f_B^u CL_{int}^u/Q$ is small, such that the extraction ratio is low ($E < 0.25$), binding is a limiting factor in clearance, and the two parameters are almost proportionally related (Equation 2, Table 21.1):

$$CL_{tot} \sim f_B^u CL_{int}^u \qquad \text{Equation 2}$$

This situation describes the traditional "restrictive" concept of binding and elimination (Figure 21-1), and many examples exist to support its validity (Wilkinson 1983). In contrast, when an organ is very efficient at eliminating a drug (*i.e.*, $Q \ll f_B^u CL_{int}^u$), then clearance is predominantly controlled by, and dependent on, organ blood flow (Table 21.1). Accordingly, the elimination process is less affected by the extent of plasma binding, and this lack of sensitivity increases with increasing free intrinsic clearance (Figure 21-1). Also, changes in binding produce a less than proportional change in extraction and total drug clearance relative to the alteration in the unbound fraction. Nevertheless, a linear relationship may be approx-

TABLE 21.1
Relationship Between Total and Unbound Drug Clearances and the Physiological Determinants of Hepatic Clearance and Route of Administration

| Route of Administration | | Clearance of Total Drug | Clearance of Unbound Drug |
|---|---|---|---|
| | General | $\frac{Qf_B^u CL_{int}^u}{Q + f_B^u CL_{int}^u}$ | $\frac{Q\,CL_{int}^u}{Q + f_B^u CL_{int}^u}$ |
| Intravenous | $CL_{int}^u >> Q$ | $Q$ | $\frac{Q}{f_B^u}$ |
| | $CL_{int}^u << Q$ | $f_B^u CL_{int}^u$ | $CL_{int}^u$ |
| Oral | All cases | $f_B^u CL_{int}^u$ | $CL_{int}^u$ |

Notes:
$Q$ = total organ blood flow

$f_B^u$ = fraction of unbound drug in blood

$CL_{int}^u$ = free intrinsic clearance of drug from hepatic intracellular water

imated when only a limited range of binding is considered. Such "non-restrictive" elimination occurs because binding equilibrium is maintained within the organ, even though unbound drug is being continuously removed along the hepatic sinusoid. This is possible because dissociation of bound drug from the binding protein is considered to occur very rapidly relative to the drug's transit time of several seconds within the organ (Gillette 1973). Unfortunately, for the vast majority of drugs this is an untested assumption since experimental measurement of rates of dissociation of drug-protein complexes is limited to a very small number of ligands. It would appear, however, that providing plasma binding is not more than about 99 percent, then hepatic extraction should not be significantly affected by binding non-equilibrium within the sinusoids (Jansen 1981). This encompasses a large number, if not the majority, of

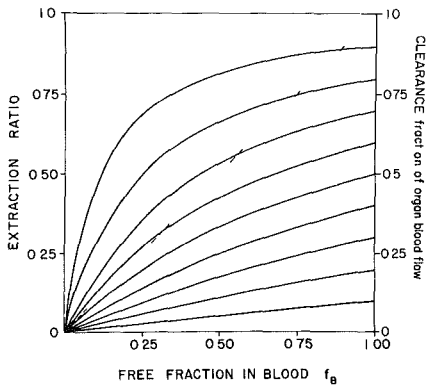


FIGURE 21-1
Effect of binding in the blood on organ extraction ratio (E). Dashed line indicates when $E = f_B^u$; below this line extraction is limited to the unbound moiety whereas above the line bound drug delivered to the organ is extracted. Each curve represents different values of $CL_{int}^u/Q$ corresponding to 10 percent stepwise changes in extraction when $f_B^u = 1$.
Source: Wilkinson and Shand 1975. (Reprinted with permission.)

drugs and it is, therefore, not unreasonable to consider the consequences of plasma binding on the blood concentration/time profile when perfusion-limited elimination is present.

Following administration of a single dose, the blood concentration of total drug is not only determined by clearance, but also by the volume of distribution. Since translocation out of the vasculature is limited to unbound drug, the volume of distribution ($Vd^{tot}$) is dependent on the extent of binding in the blood (Equation 3).

$$Vd^{tot} = V_B + \sum_{i=1}^{n} \frac{f_B^u}{f_{T,i}^u} \cdot V_{T,i} \qquad \text{Equation 3}$$

where $V_B$ is the blood volume, $V_{T,i}$ is the volume of tissue water for each individual (i-th) tissue, $f_{T,i}^u$ is the unbound fraction in the i-th tissue, and $f_B^u$ is the unbound fraction in the blood (Gillette 1971; Wilkinson and Shand 1975). Accordingly, distribution volume decreases linearly as blood binding increases (Wilkinson and Shand 1975; Tozer 1981).

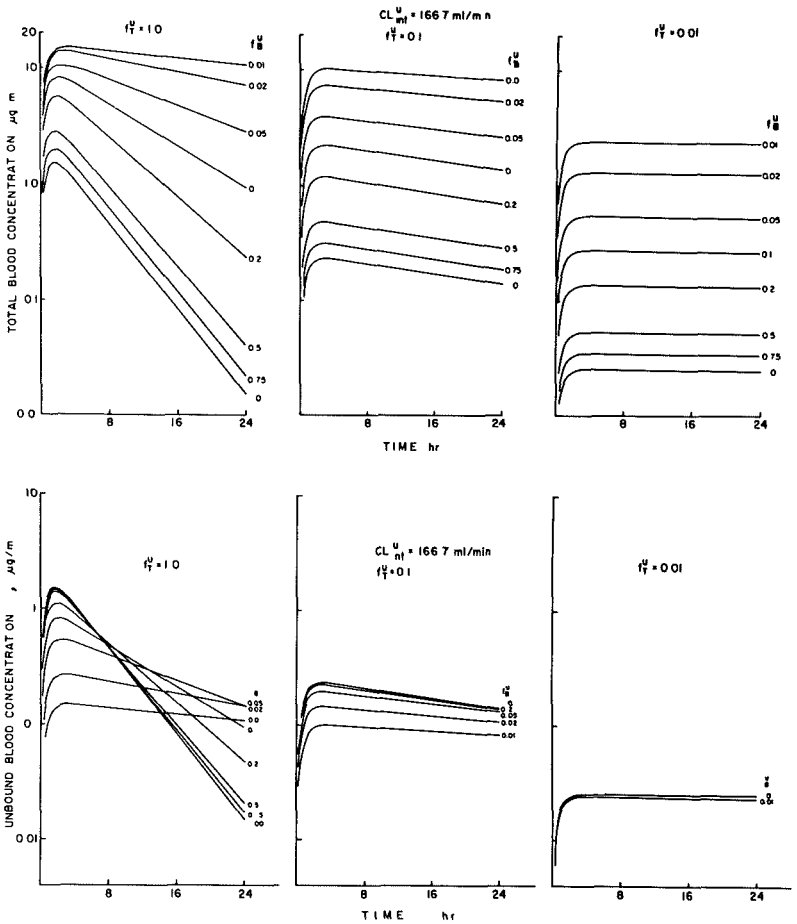


FIGURE 21-2
Effect of binding in the blood ($f_B^u$) on the blood concentration/time curves of total (upper panels) and unbound (lower panels) drug after oral administration, according to the "well-stirred" model of hepatic elimination assuming that this is the only route of removal. Three examples of drug are indicated with the same small free intrinsic clearance ($CL_{int}^u$) but different total volumes of distribution represented by $f_T^u$, the unbound fraction of drug in the lumped extravascular tissue (see Equation 3).

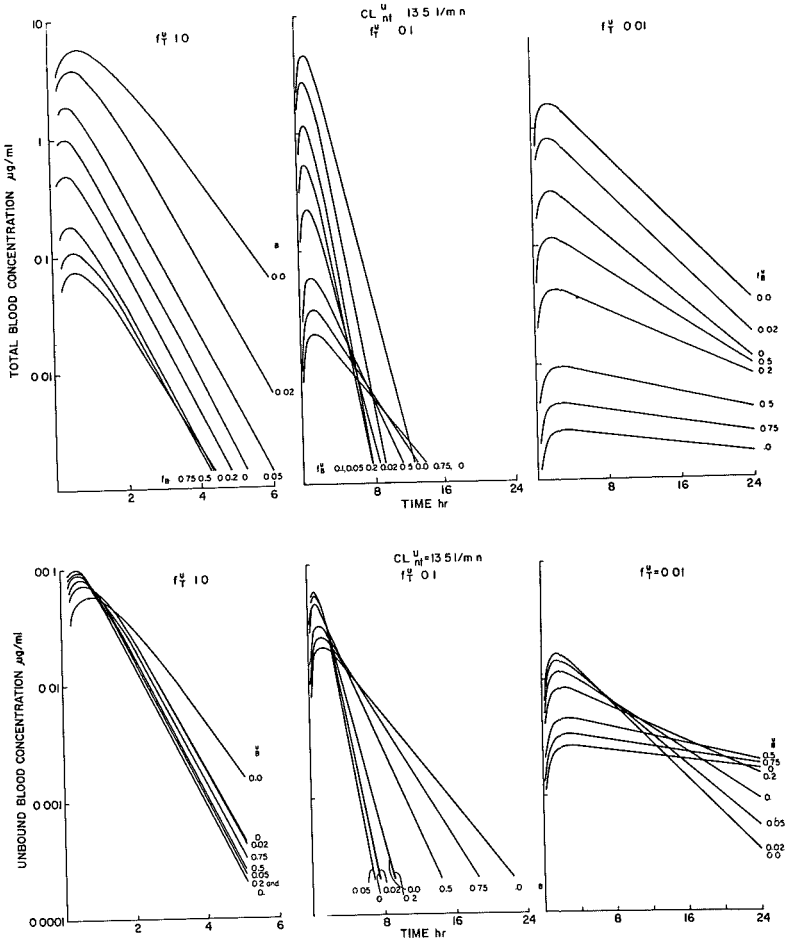


FIGURE 21-3
Effect of binding in the blood ($f_B^u$) on the blood concentration/time curves of total (upper panels) and unbound (lower panels) drug after oral administration, according to the "well-stirred" model of hepatic elimination assuming that this is the only route of removal. Three examples of drug are indicated with the same high free intrinsic clearance ($CL_{int}^u$) but different total volumes of distribution represented by $f_T^u$, the unbound fraction of drug in the lumped extravascular tissue (see Equation 2).

Since a drug's elimination half-life is proportional to the volume of distribution, but inversely related to clearance, both of which are determined by the unbound fraction in the blood, the consequences of binding on this parameter are complex. In addition to the unbound fraction, the free intrinsic clearance and extent of tissue binding need to be considered (Wilkinson and Shand 1975; Tozer 1981). For a drug eliminated in a "restrictive" fashion (i.e., low $CL_{int}^{u}$ and/or $f_B^u$), an increase in the unbound fraction in the blood increases both total clearance and volume of distribution, the net effect depending on the relative magnitude of these changes. For example, if tissue binding is not extensive so that $Vd^{tot}$ is relatively small, the clearance change predominates and half-life shortens with an increase in $f_B^u$. Accordingly, after oral drug administration, total drug concentrations are higher and decline more slowly the smaller the unbound fraction in the plasma (Figure 21-2). In contrast, the unbound, and presumably pharmacologically active, levels are lower and decline more slowly as $f_B^u$ becomes smaller (Figure 21-2), since the clearance of unbound drug ($CL_{tot}/f_B^u \sim CL_{int}^u$) remains constant. On the other hand, when distribution is extensive ($f_T^u \rightarrow 0$), then the elimination half-life is increasingly determined by the degree of tissue binding (Gibaldi, Levy, and McNamara 1978), and plasma binding has a minimal effect on the rate of drug removal from the body. At the other extreme, when free intrinsic clearance is high relative to hepatic blood flow, an increase in the plasma unbound fraction has essentially no effect on total clearance until $f_B^u$ becomes relatively small (Figure 21-1), but distribution is still affected. Therefore, following oral administration of a drug with a high free intrinsic clearance, peak concentrations of total drug are higher as the unbound fraction becomes smaller, and at the same time the elimination half-life shortens (Figure 21-3) as, for example, with propranolol (Evans, Nies, and Shand 1973). Significantly, the initial blood concentrations of unbound drug increase as the unbound fraction decreases (Figure 21-3); i.e., an effect opposite to that observed with drugs exhibiting "restrictive" elimination. For drugs which are not so effectively eliminated, i.e., an intermediate extraction ratio, a biphasic relationship exists such that increased plasma binding can shorten the half-life or prolong it, depending on the relative degrees of plasma and tissue binding.

A difference in behavior between "restrictively" and "non-restrictively" eliminated drugs is also present when chronic dosing is considered (Table 21.1). When free intrinsic clearance is small, then the clearance and average steady-state blood level of unbound drug are independent of binding in the blood, regardless of the route of administration, and total concentrations become smaller as the unbound fraction increases. On the other hand, for a drug with a high

free intrinsic clearance, total clearance and steady-state drug concentrations after intravenous administration are not affected by binding, but unbound levels increase as $f_B^u$ increases (Shand, Mitchell, and Oates 1975). With oral dosing, however, the situation is the same as for a "restrictively" eliminated drug, i.e., steady-state total concentrations are reduced as the unbound fraction increases, but unbound drug concentrations are unaffected. Unfortunately, few experimental studies have been reported to confirm these theoretical effects of plasma binding on the blood concentration/time profiles of drugs with high hepatic free intrinsic clearances, especially with regard to different routes of administration and duration of dosing.

Another quantitative approach to hepatic elimination is the "sinusoidal" or "parallel-tube" model (Winkler et al. 1974, 1979). Rather than uptake being a function of the organ's "equilibrium" concentration as in the "well-stirred" model, this alternative model assumes that there is an exponentially declining concentration gradient along the sinusoid. Again, intra-organ binding equilibrium is assumed to be maintained within the sinusoid. Accordingly, total clearance and extraction are described by Equation 4:

$$CL_{tot} = Q(1 - e^{-f_B^u CL_{int}^u / Q}) \qquad \text{Equation 4}$$

In general, both models predict the same type of overall relationships for clearance and other pharmacokinetic parameters (Pang and Rowland 1977). This is particularly so for drugs with low free intrinsic clearances and/or high plasma binding. Significant differences are, however, predicted for drugs with high hepatic extraction ratios, i.e., high $CL_{int}^u$, particularly following oral drug administration (Pang and Rowland 1977). With regard to plasma binding, a clear discriminator between the two models appears to be the relationship of oral clearance of unbound drug and the extent of binding (Morgan and Raymond 1982). In the "sinusoidal" model this clearance increases as the unbound fraction increases, whereas in the "well-stirred" model these two determinants are independent. Experimental studies to determine the validity of one or the other models using perturbations in binding have been relatively few and provided conflicting findings. For example, Jones et al. (1984) found that after portal venous infusion the steady-state concentration of unbound propranolol in the perfused rat liver preparation was constant as $f_B^u$ was changed sevenfold, a result consistent with the "well-stirred" model. On the other hand, Rowland et al. (1984) concluded from similar studies with diazepam that the "sinusoidal" model provided a better explanation for the data, and in vivo studies with intravenous

quinidine in the rabbit were unable to discriminate between the two models (Guentert and Øie 1980). Similar conflicting data in support of one or the other models have been obtained when other determinants, such as hepatic blood flow, have been perturbed (Bass 1983; Jones et al. 1984; Morgan 1983; Wilkinson et al. 1978). A number of technical reasons have been advanced to explain such contradictory data, including the possibility that both models are an oversimplification and neither accurately reflects the actual biological events.

More complicated models, such as the distributed-sinusoidal model have been developed in which the lengths of the sinusoids and their eliminating ability are not assumed to be uniform (Bass, Robinson, and Bracken 1978; Forker and Luxon 1978). However, studies appropriately designed to discriminate between these and the simpler models have not yet been reported. The effects of saturable plasma binding on hepatic elimination (Huang and Øie 1984) and also blood concentration/time profiles (Øie, Guentert, and Tozer 1980) have also been considered. There is also the probability that the functional heterogeneity of the liver may preclude a single universal model applicable to the elimination of all substrates regardless of the mechanisms involved. It is also important to recognize that experimental perturbations used to discriminate between the models such as altered plasma binding or blood flow may produce functional change in the liver. For example, the uptake of sulfobromophthalein by the isolated perfused rat liver in the absence of albumin results in a large tissue concentration ratio between zone 1 and zone 3 of the hepatic acinus (Gumucio et al. 1984). However, in the presence of albumin in the perfusate this zonal gradient disappears because of decreased extraction by the hepatocytes first exposed to the incoming drug. Thus, the lack of, or the presence of low binding results in the liver behaving in a fashion consistent with the "sinusoidal" model, but when extensive binding is produced by albumin there is a change to the "well-stirred" model.

## ALBUMIN CATALYZED DISSOCIATION AND HEPATIC ELIMINATION

Perfusion-limited hepatic elimination is based on the conventional concept that only unbound drug in the sinusoidal blood is available for uptake and, as this occurs, the protein-ligand complex dissociates to maintain the binding equilibrium. However, the extraction ratio of a number of extensively albumin bound compounds, including fatty acids, organic anions, and bile acids, is higher than the theoretical limit given by the complex's dissociation rate constant and the transit time of albumin through the liver. An increasing body

of evidence suggests that this situation may be accounted for by interaction of the albumin-ligand complex to a specific recognition (receptor) site on the hepatocyte plasma membrane which facilitates subsequent uptake (Weisiger, Gollan, and Ockner 1982; Ockner, Weisiger, and Gollan 1983).

Initial evidence of the involvement of bound ligand in organ uptake was obtained with fatty acid (oleate) extraction in the perfused rat liver (Weisiger, Gollan, and Ockner 1981). Studies in which the albumin concentration in the perfusate was maintained constant and the oleate concentration was varied to provide a ten-fold range in the oleate:albumin ratio indicated that uptake was linearly related to the perfusate oleate level (Figure 21-4). However, when the albumin: oleate ratio was fixed at 1:1 and the concentrations of both were varied together, the uptake was nonlinear and could be essentially described by saturable, Michaelis-Menten type kinetics. Importantly, in neither experimental design were the uptake rates consistent with the calculated equilibrium concentration of unbound oleate. Further-

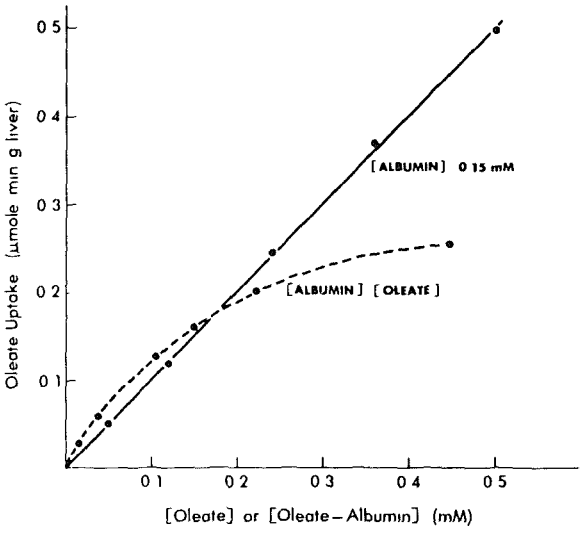


FIGURE 21-4
Oleate uptake by the perfused rat liver at fixed albumin (1 percent) and variable oleate concentrations (solid line), and for simultaneous variation of both oleate and albumin at a fixed 1:1 molar ratio (dashed line). The saturation of the uptake rate seen with increasing concentrations of the fixed molar ratio oleate-albumin complex occurs at rates well below those attained with variable oleate and fixed albumin, thus supporting the model for hepatic uptake shown in Figure 21-5. Source: Weisiger, Gollan, and Ockner 1982. (Reprinted with permission.)

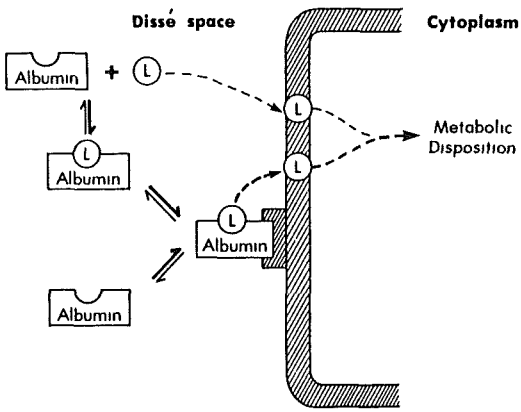


FIGURE 21-5
Albumin catalyzed dissociation model of hepatic elimination. Uptake of ligand predominantly occurs by a saturable interaction of the albumin-ligand complex with binding sites on the hepatocyte or to a lesser extent by spontaneous dissociation and diffusion of unbound ligand (L) to the cell surface.
Source: Weisiger, Gollan, and Ockner 1982. (Reprinted with permission.

more, the apparent saturation did not appear to reflect any limitation of an intrinsic uptake or elimination process since it occurred at rates well below those attained in those studies in which no saturation occurred when the albumin concentration was constant. Similar findings have also been observed for the hepatic uptake of bilirubin and sulfobromophthalein (Weisiger, Gollan, and Ockner 1980, 1982).

These results are consistent with a model (Figure 21-5) in which a transient but saturable interaction occurs between the albumin-oleate complex and the hepatocyte surface, resulting in the uptake of the oleate and release of the albumin. As the concentration of the complex increases, uptake also increases until the availability of albumin-binding sites on the cell surface becomes limiting, leading to a saturation effect. It is further speculated that the interaction leads to enhanced dissociation of the albumin-ligand complex, thus allowing uptake at a rate above that occurring spontaneously in bulk-solution (Ockner, Weisiger, and Gollan 1983). The mechanism of this facilitation is not known, but could involve a conformational change in the albumin molecule, perhaps resulting from the interaction with the cell surface, which alters the albumin-ligand binding affinity.

A similar model has been independently concluded using a different experimental approach to study the extraction of taurocholate

(Forker and Luxon 1981) and rose bengal (Forker et al. 1982; Forker and Luxon 1983) by the isolated perfused rat liver. For example, the extraction ratio of taurocholate at a perfusate albumin concentration of 0.5 g/dl is 97 percent. Increasing the albumin concentration ten-fold reduces the concentration of unbound bile acid by a factor of five but produces only a 50 percent reduction in the uptake rate constant. Kinetic analysis suggests that uptake is predominantly due to an interaction between albumin-bound drug and the hepatocyte membrane rather than the unbound taurocholate.

Additional evidence for the presence of cell surface binding sites for albumin, which may be involved in the hepatic uptake of highly albumin-bound ligands, comes from direct studies. Albumin ($^{125}$I-labeled) reversibly, but specifically, binds to rat hepatocytes in a saturable fashion (Weisiger, Gollan, and Ockner 1981). Kinetic analysis indicates the presence of single, relatively high affinity, low capacity binding site, equilibrium dissociation constant between $10^{-5}$ and $10^{-6}$ M, and a low affinity, non-saturable site. Each cell contains about $10^7$ binding sites which represent about 1-8 percent of the total hepatocyte surface. Interestingly, similar values have been independently derived from the analysis of data on the extraction of rose bengal by the perfused rat liver (Forker and Luxon 1983).

Consistency of experimental observations with a mathematical model does not prove that an albumin binding site is necessarily involved in the hepatic uptake of highly bound ligands. Further validation will require additional evidence, including the nature of the putative binding site; the mechanism of the albumin-binding site interaction and the catalyzed-dissociation process; the physiological regulation of the number and/or affinity of the binding sites, and the possibility of similar processes in other organs. Nevertheless, the increasing evidence that the albumin-ligand complex may be directly involved in hepatic uptake and elimination is a provocative challenge to the established dogma that limits such transport to the unbound drug present in the sinusoid.

## DISSOCIATION-LIMITED HEPATIC ELIMINATION

Almost all studies of hepatic uptake have implicitly or explicitly assumed that dissociation of bound ligand from the binding protein occurs sufficiently rapidly relative to organ transit time that binding equilibrium is maintained throughout the sinusoid. However, as previously indicated, the experimental evidence to support this assumption is extremely meager. Theoretical analysis (Jansen 1981) suggests that the rate of dissociation of the binding complex could limit hepatic removal of compounds that are highly bound (> 99 percent)

and have a high free intrinsic clearance such that the extraction ratio is greater than 95 percent when binding is absent. Again, experimental studies are very limited, but nevertheless, it is probable that under certain circumstances, dissociation-limited uptake may be important. In this regard, recent observations on the elimination of sulfobromophthalein in the skate are particularly noteworthy, especially with respect to the concept of bound drug uptake by the putative cell surface, albumin binding site (Weisiger et al. 1984).

The uptake of sulfobromophthalein by the perfused skate liver follows Michelis-Menten kinetics under conditions where the albumin concentration is maintained constant and the ligand level is progressively increased. This reflects the involvement of a carrier-mediated uptake mechanism similar to that in mammalian species, and the Vmax and Km are independent of the albumin concentration. Interestingly, a similar type of uptake kinetics was observed when the albumin and sulfobromophthalein concentrations were simultaneously varied at a fixed molar ratio. However, the resulting apparent kinetic constants were markedly different from those values obtained under conditions of constant albumin concentration and were dependent on the molar ratio. This latter observation is similar to that observed in the perfused rat liver for bilirubin, sulfobromophthalein, and fatty acids (Weisiger, Gollan, and Ockner 1980, 1981) using an identical experimental design, and from which it was concluded that albumin mediated uptake occurs by means of a specific cell surface binding site (Figure 21-4). Significantly, however, elasmobranchs, such as skates and sharks, lack serum albumin and by inference, a specifically evolved albumin binding site. It is unlikely, therefore, that this latter mechanism of uptake can be involved. Instead, the data obtained in the skate has been interpreted as indicating that in this situation, hepatic uptake is rate-limited by the dissociation of bound sulfobromophthalein from albumin (Weisiger et al. 1984).

Binding equilibrium within the sinusoid will only exist when the rate of unbound ligand removal is sufficiently small that it does not significantly alter the unbound concentration. This occurs only if the rate at which newly dissociated unbound ligand is rebound by albumin is much greater than the rate at which the same unbound ligand is removed by the liver. Accordingly, the concentration of albumin is a critical determinant of whether binding equilibrium is present within the sinusoid. Furthermore, the higher the uptake rate constant, the greater the albumin concentration required to maintain binding equilibrium. Conversely, the higher the association rate constant, the lower the albumin level required to maintain equilibrium. Thus, apparent saturation kinetics may arise through a gradual transition from dissociation-limited to equilibrium conditions resulting from an increasing albumin concentration when the molar ratio

is fixed, but ligand and albumin levels are simultaneously increased. Furthermore, a linear relationship would be expected between total ligand concentration and uptake rate when the albumin concentration is fixed. Additional theoretical analysis of a general model (Weisiger 1985) indicates that hepatic extraction and clearance of a ligand may be limited by the rate of plasma flow, dissociation from albumin, influx into the hepatocyte, free intrinsic clearance, or any combination of these factors. The affinity of reversible albumin binding and the concentration of the binding macromolecule are particularly important and strongly determine which one or more of these steps is rate limiting for any particular ligand and condition. Particularly noteworthy is the fact that binding equilibrium exists within the sinusoid only when the albumin concentration is above a certain value specific to the ligand.

These recent findings clearly indicate previously unrecognized complexities in the interpretation of the effects of plasma binding on hepatic uptake/elimination, since the rate-limiting process may change as the concentration of binding macromolecule is altered, and this phenomenon may be ligand dependent. Such factors may account for certain conflicting findings that have been reported (Jones et al. 1984; Rowland et al. 1984). The observations also raise questions concerning the involvement of a cell surface, albumin-binding site in hepatic uptake, since this putative mechanism is based in part upon similar albumin-dependent kinetics. Discriminative studies are obviously required, but current evidence would suggest that uptake by the elasmobranch liver is somewhat different and less efficient than from the rat liver and some form of catalyzed dissociation must exist in order to account for the observed organic anion uptake rates in the latter (Weisiger et al. 1984).

## CONCLUSIONS

Recent years have seen a greater appreciation and understanding of the quantitative consequences of plasma binding on drug disposition, and hepatic elimination in particular. In general, it is now possible to fairly accurately predict the effects of perturbed binding on distribution, clearance and blood/plasma concentration/time profiles. However, less confidence exists at the organ level, which reflects a lack of understanding of intra-organ events. Several alternative models of hepatic elimination exist, for example, and involve completely different conceptual approaches regarding the role of unbound and bound drug in the initial uptake process. Discrimination between these alternatives will be challenging and no single model may be appropriate for all ligands. However, such studies are likely

to provide new and fundamental insights of broad physiological and pharmacological significance.

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