The influence of binding to albumin and α_1-acid glycoprotein on the clearance of drugs by the liver

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Introduction

The influence of plasma protein binding on hepatic clearance has long been neglected. Many kinetic studies in animals and man for experimental or diagnostic reasons have been performed in the past to investigate the hepatic transport function for endogenous and exogenous compounds. Often conclusions were drawn with regard to liver function or kinetic interactions of such agents, without taking into account possible changes in the extent of protein binding of the agents studied. Nevertheless, many drugs undergoing hepatic metabolism or hepato-biliary transport are to a large extent protein-bound. This is partly related to their amphipathic character that favours hepato-biliary transport and/or metabolism but also may promote association with plasma proteins.[1]

In plasma at least two different proteins are responsible for most of the binding: albumin and α_1-acid glycoprotein (orosomucoid) (Table 1). Roughly speaking the first protein, having a relatively high plasma concentration (4.0% or 600 μM), binds acidic (anionic) drugs,[1 2] the second, at much lower concentrations (0.1% or 22 μM), binds predominantly basic (cationic) drugs, including tertiary[1 3 4] and quaternary amines.[3] Both proteins are synthesized in the liver and especially during chronic liver diseases and/or loss via the urine with renal disease, plasma albumin levels can be abnormally low. During acute-phase reactions such as inflammation, tumours, and burns, the concentration of α_1-acid glycoprotein in plasma may be largely elevated through increased hepatic synthesis.[2-4]

Drug binding to these proteins is a saturable phenomenon and can be characterized by determining the number of binding sites (n) per protein molecule as well as the affinity for these binding sites.[1] The unbound fraction of a drug (*fu*) will therefore be determined by the total plasma concentration of the drug and protein, the capacity of binding of the protein as well as the affinity for the particular binding sites. The unbound fraction (*fu*) times the total drug concentration (*C*) yields the unbound concentration (*Cu*). For anionic drugs separate classes of binding sites are present on the albumin molecule, although such binding sites for acidic drugs often have an overlapping substrate specificity. Saturation of these binding sites will occur for albumin only after relatively high doses of drugs, whereas α_1-acid glycoprotein can already be saturated at low concentration of drugs.[1]

For several reasons, changes in the unbound

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Metabolism
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Protein binding

Abstract

The liver is a major site for synthesis and catabolism of plasma proteins. Albumin has various binding sites for anionic drugs, α_1-acid glycoprotein possesses a single binding site for cationic drugs. In spite of extensive protein binding, the liver can efficiently remove drugs from the circulation. Intrahepatic dissociation of the drug–protein complex may involve dissociation-limited debinding under non-equilibrium conditions or surface interaction-facilitated dissociation phenomena. During liver or renal disease and acute-phase conditions plasma protein binding of drugs may be affected. Changes in the unbound drug fraction do not always result in proportional changes in clearance or distribution volume. Potential changes in the unbound concentration in steady-state as well as the fluctuations in total plasma levels depend on the extent of protein binding of a drug, the relative change in the unbound drug fraction, type of clearance, the size of the distribution volume, route of administration as well as concomitant changes in intrinsic (cellular) clearance function. Optimization of dosage regimens for certain drugs and interpretation of liver function tests with diagnostic dyes may largely benefit from determination of the unbound rather than the total concentration of the drugs involved.

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fraction of drugs in the plasma are not necessarily linearly related to variation in hepatic clearance. For instance, factors other than protein binding, such as organ blood flow, can be rate-limiting while *fu* of the drug within the vascular space of the liver may exceed that in the general circulation due to intrahepatic dissociation of the drug–protein complex. Changes in *fu* due to competitive interaction of drugs at the level of plasma proteins can be estimated on the basis of the concentration of the two drugs as well as the respective binding affinities using appropriate equations.[1] However, due to uncertainties as to the presence of endogenous compounds and influence of (often not well characterized) high-capacity, low-affinity binding sites, it is preferable to determine the *fu* in plasma directly by ultrafiltration, ultracentrifugation or dialysis of plasma. This requires a proper reproducibility of such methods and also a sensitive bioanalysis of the drug.

For instance, the relatively low dosed indometacin is about 99.8% bound to plasma protein and thus *fu* is in the order of 0.002. It is clear that even a slight displacement, for instance resulting in a new binding percentage of 99.0, will increase *fu* fivefold. Consequently especially for drugs with binding over 90%, displacement interactions or changes in protein concentration will produce large relative changes in *fu*. The influence of such changes in plasma protein binding on hepatic clearance can be predicted by using various perfusion models of the liver (see Table II). For instance, the 'well stirred' model, picturing the liver as an optimally mixed compartment operating at the outflow (venous) drug concentration, or the 'parallel tube' model, assuming an exponential decrease of drug concentration along the portal to central vein axis, may provide different estimations of clearance at a variation of *fu*, especially for drugs with a high hepatic extraction.

TABLE I
*Characteristics of two major drug binding proteins in blood plasma**

	Albumin	α_1-Acid glycoprotein (orosomucoid)
Molecular weight	65 000	44 000
Isoelectric pont	4.8; 5.6	2.7
Sugars (%)	< 2	40
Plasma level	600 μ*M*	15 μ*M*
Increase by	exercise benign tumours hypothyreoidism psychiatric disorders	stress inflammatory diseases burns, trauma, surgery myocardial infarction tumours
Decrease by	nephrotic syndrome chronic liver disease pregnancy age gastro-intestinal disease malignant tumours	liver cirrhosis nephrotic syndrome oral contraceptives
Drugs bound		
– category I	bilirubin coumarins pyrazolinones thiazide diuretics	quinidine, disopyramide local anaesthetics narcotic analgesics psychotropic drugs beta-blocking agents anticholinergic agents
– category II	tryptophan benzodiazepines arylacetic analgesics sulfobromphthalein ethacrynic acid hypolipidaemic agents	

*Pathological conditions inducing positive and negative changes in the protein concentrations are indicated. Albumin contains two different classes of binding sites for anionic drugs, one class of binding site is assumed to be present on α_1-acid glycoprotein.

Protein binding and clearance

Variations in the unbound fraction of the drug in plasma may also alter the clearance of drugs by the liver.[1 5] One factor is the dependence on a possible rate limitation in the clearance process. If clearance is relatively low compared with hepatic blood flow, clearance may linearly vary with *fu* since the driving force for the metabolism and excretion processes is the free concentration of the drug. However, if clearance is high compared with blood flow, the latter may become rate-limiting, and an increase in *fu* will only moderately affect hepatic clearance. The equations describing the relation of *fu* with clearance

TABLE II
*Implications of protein binding for drug clearance and distribution**

Clearance

– 'well stirred' model $$CL_H = Q_H \frac{fu \cdot CL_{int}}{Q_H + fu \cdot CL_{int}} \quad (eq.1)$$

– 'parallel tube' model $$CL_H = Q_H\,(1 - e^{-fu \cdot CL_{int}/Q_H}) \quad (eq. 2)$$

Distribution $$V = V_p + V_T \cdot fu/fu_T \quad (eq. 3)$$

*CL_H = hepatic clearance; Q_H = liver blood flow; fu = fraction unbound in blood; CL_{int} = intrinsic clearance (expressing the cellular activity in absence of limitation by blood flow and protein binding); V = distribution volume; V_p = plasma volume; V_T = volume of tissue to which the drug distributes; fu_T = fraction unbound in tissue.

for two commonly used perfusion models are depicted in Table II. Both equations refer to steady-state conditions in hepatic clearance: a situation in which equilibrium between plasma and liver compartments is reached and per unit time as much drug is cleared from the body as is entering the body.

It should be mentioned here that the initial rate of distribution of a drug to the liver after an intravenous bolus injection can also be influenced by protein binding and in principle the above-mentioned concepts can also be applied to this process.[6] It follows that distribution to the liver, for many drugs being a very rapid process, will have the tendency to be more limited by blood flow and less influenced by changes in protein binding than the actual clearance process (either metabolism or biliary excretion or both).[6,7] From these considerations one can conclude that variations in protein binding will be reflected in changes in hepatic disposition of drugs, but the extent of such a change is not only dependent on the magnitude of change in *fu*, but also on the type of clearance of the particular drug. If this aspect is not taken into account, major misinterpretations in the estimation of liver function can be made.

For instance, if due to liver disease the albumin concentration is abnormally low, the decreased intrinsic clearance function may be partly or even completely masked by an increased *fu*. This effect may even be more prominent if under these pathological conditions endogenous compounds accumulate in plasma and compete with the drug for binding to plasma proteins. Also, drug interactions on the cellular level may not be clearly expressed if the drugs mutually compete for binding to plasma proteins and changes in *fu* are more pronounced than interactions on the level of hepatocellular metabolism or excretion.[7]

For these reasons it should be preferred to determine the free instead of the total concentration of drugs to characterize liver function for diagnostic purposes or for the characterization of kinetic drug interactions at the levels of plasma proteins or cellular disposition. For example, hepato-biliary clearance of anionic dyes was shown to be largely dependent on the plasma albumin concentration as observed in man,[8] intact animal,[9] isolated perfused rat liver[6,7] and also in isolated hepatocytes.[10] Under these various conditions clearance seems inversely related to the albumin concentration. The same can be concluded for metabolism of drugs by the liver as has been reported by Rowland.[5]

Protein binding and volume of distribution

In the intact animal lower binding to albumin or other plasma proteins also leads to an increase in distribution volume of the drug. The effect of changes in free fractions *fu* on the distribution volume can be described by equation 3 of Table II. If the plasma volume (V_p) is small compared with the total distribution volume (V), V_p in equation 3 can be neglected and V changes linearly with *fu*.

A twofold increase in *fu* of a drug with a capacity-limited type of hepatic clearance would lead to a twofold increase both in apparent clearance and distribution volume. This implies that the rate of elimination (for instance expressed as $t_{1/2}$) would be unchanged. However, for a drug with a flow-limited type of clearance, distribution volume would increase twofold, but in contrast clearance would be unchanged since total hepatic blood flow cannot be increased further. Consequently, an increase in *fu* in such a case would lead to an increased $t_{1/2}$ of elimination.[1,5] This illustrates not only that one should not use parameters that depend on distribution volume (such as $t_{1/2}$) for characterizing clearance function, but also that diagnosis of abnormal transport function would be improved by measuring the unbound drug concentration instead of total concentration.[7,8,11] At the same time occurrence of non-linear kinetics should be considered if *fu* is increased.

Protein binding and hepatic storage of drugs

Differences in protein binding should also be taken into account if one compares transport capabilities in isolated livers or isolated hepatocytes with liver function in the intact animal.[12] A study of Schwartz and Klaassen comparing uptake rate of sulfobromphthalein (BSP) and its glutathione (GSH) derivative BSP-GSH suggested that BSP-GSH has a much lower uptake rate than BSP.[13] This study was done in isolated hepatocytes without albumin in the incubation medium. In isolated perfused rat livers with albumin-containing perfusion medium,[14] however, uptake of BSP-GSH in the liver occurred more rapidly than for BSP. This discrepancy is resolved if the large difference in protein binding is taken into account: BSP-GSH is bound to albumin 10-100 times less than BSP.[15]

A decrease in protein binding of indocyanine green (ICG) during infusion of non-protein plasma expanders in cats may very well explain the increase in hepatic clearance as observed by the authors.[16] If distribution of a drug is mainly restricted to plasma and liver, as is for example the case with ICG, equation 3 (Table II) simply demonstrates that a change in *fu* will lead to an increase in hepatic distribution volume and thus in the amount of ICG in the liver at a certain plasma concentration. This demonstrates that hepatic storage of drugs, apart from saturable membrane transport, is also determined by relative binding in the plasma and liver compartments.[7]

Figure 1 shows that if albumin is added to a perfused liver, preloaded with dibromosulfophthalein (DBSP), redistribution to the plasma occurs until a new steady-state is reached.[7] Such a storage function of the liver can pharmacokinetically

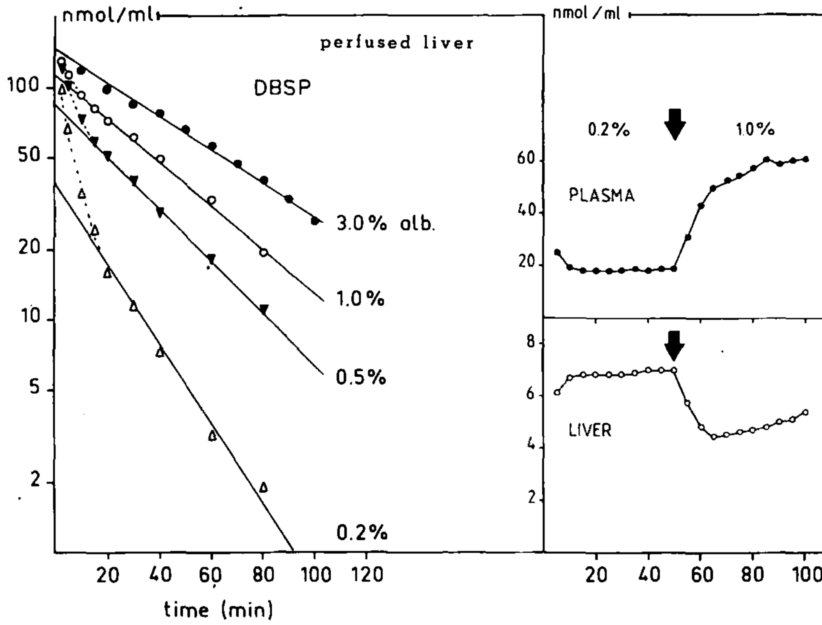

FIGURE 1
Influence of albumin on hepatic uptake and efflux of the organic anion dibromosulfophthalein (DBSP) in isolated perfused rat liver. Single doses were given at various albumin concentrations in the perfusion medium (left panel). Continuous infusion in a perfusate with 0.2% albumin leads to steady-state levels. Addition of albumin to a concentration of 1.0% leads to redistribution of DBSP from liver to perfusion medium

be quantified by analysing the biexponential disappearance curves under first-order conditions with a two-compartmental model or by constant infusion of such agents at various rates exceeding the maximal biliary excretion rate.[6 7] The latter method, however, is risky due to extrahepatic distribution and elimination, potential toxicity of the compounds and saturation of membrane transport steps other than biliary excretion, so that assumed linear kinetic conditions no longer apply.[7] This may especially be a problem in individuals with liver disease and abnormal plasma protein levels.

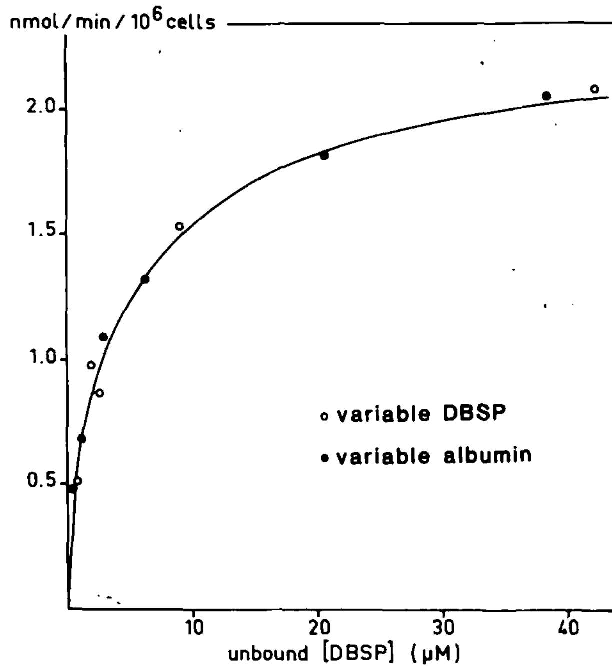

FIGURE 2
Initial uptake rate of dibromosulfophthalein (DBSP) in isolated hepatocytes as a function of the unbound concentration in the incubation medium. The initial DBSP concentration was varied at a fixed albumin concentration (○) or albumin was varied at a fixed DBSP concentration (•). Identical curves were obtained in spite of largely varying albumin/DBSP concentration ratios

Figure 2 shows that uptake rate of the organic anion DBSP in isolated hepatocytes is largely influenced by albumin concentration in the incubation medium. If in such hepatocyte studies the DBSP concentration is varied at a fixed albumin concentration or in contrast albumin is changed at a fixed DBSP concentration, identical uptake curves are found if unbound concentration is related to initial uptake rate.[12] Figure 2 shows a typical Michaelis-Menten type of uptake for both experimental conditions. This implies that at very different concentrations of albumin, uptake rate is identical provided that the unbound concentration is the same. Albumin in *in vitro* conditions seems to have no other role than to reduce the free concentration as the driving force for uptake. However, the situation in the flow-dynamic system of the intact organ may be quite different since hepatic blood flow rather than cellular transport function may be the rate-limiting step in distribution to the liver.

Dissociation of drug–protein complexes in the liver

The classical concept that the rate of hepatic removal of a ligand is determined solely by the unbound concentration and that the protein-bound fraction in that respect is inert, may not necessarily be valid for any compound. It was already suggested by Baker and Bradley in 1966 as well as by Bloomer *et al.* in 1972 that dissociation of tightly bound substances such as BSP and bilirubin might be too slow to adequately replace the drug that is cleared

during passage through the liver.[15 17] Since the liver is able to extract compounds which are more than 99% bound with an extraction fraction of up to 80%, these authors suggested that, apart from progressive spontaneous dissociation within the liver sinusoids and space of Disse, direct interactions of the protein–drug complex occur at the plasma membrane, leading to a facilitated dissociation of the complex (surface-mediated dissociation). The latter hypothesis underwent a revival through more recent and independent observations that the hepatic uptake kinetics of taurocholate[18] and of fatty acids[19] were better related to the bound than to the unbound fraction. In essence, this idea was based on the findings that increasing oleate and albumin at a constant molar ratio, and theoretically leaving *Cu* unchanged, resulted in saturation kinetics in the uptake process.

This was tentatively explained by saturation of specific binding sites for albumin at the sinusoidal plasma membrane (Fig. 3). In addition to taurocholate, for rose bengal and thyroxine it was found that a marked decrease in free fraction, due to addition of albumin, resulted only in a relatively small change in hepatic uptake rate.[20-22] Such a phenomenon can also be inferred from experiments with DBSP after single injection[6] and constant infusion.[7] These studies, however, do not necessarily imply that albumin facilitates uptake of organic anions as was suggested in some titles of the related papers.[18 19 23] These observations rather brought up the possibility that the liver possesses a debinding mechanism to compensate for the inhibiting effect of albumin on uptake rate of these agents. In this respect it is of interest that hepatic uptake of organic anions such as BSP, bilirubin and taurocholate in an albuminaemic species, in which binding occurs to other plasma proteins, is quite normal.[9 24]

Interpretation of the above-mentioned non-linear relation between unbound fraction and uptake rate is clearly multifactorial and may depend on the type of compound under study as well as the experimental conditions. First, data on protein–drug dissociation rate *in vitro* under equilibrium conditions cannot always be extrapolated to the events occurring in a flow-dynamic system such as the liver. Very likely non-equilibrium conditions in dissociation of drug–protein complexes may occur.[25] In some cases this process may even become rate-limiting (Fig. 3) and will give the impression of saturation of uptake if the concentration of the protein–drug complex is increased.[25] In addition, albumin promotes efflux of certain drugs from the liver[7 26] and thereby affects net hepatic uptake in the various zones of the hepatic acinus at non-steady-state conditions.[27] Also increase in the free concentration of drugs may not always be linearly reflected in uptake rate, since hepatic plasma flow may become rate-limiting or saturation of membrane transport can occur and such factors should be incorporated in the analysis of these phenomena.[7 18 23]

Alternatively spontaneous dissociation of the drug from the protein *in vivo* may be facilitated due to competition with endogenous metabolites or membrane components escaping from the liver in the space of Disse. Even without the latter competitive debinding effect, spontaneous dissociation due to efficient progressive removal of the drug by the transport system may be rapid enough for explaining the disproportionate relation between changes in *fu* and uptake rate, in particular for compounds with a moderate affinity for the plasma protein.[25] Nevertheless, surface-mediated facilitation of drug–protein dissociation is certainly not excluded and may be of importance for drugs with poor water solubility or extremely high affinity for albumin, such as fatty acids and bilirubin.

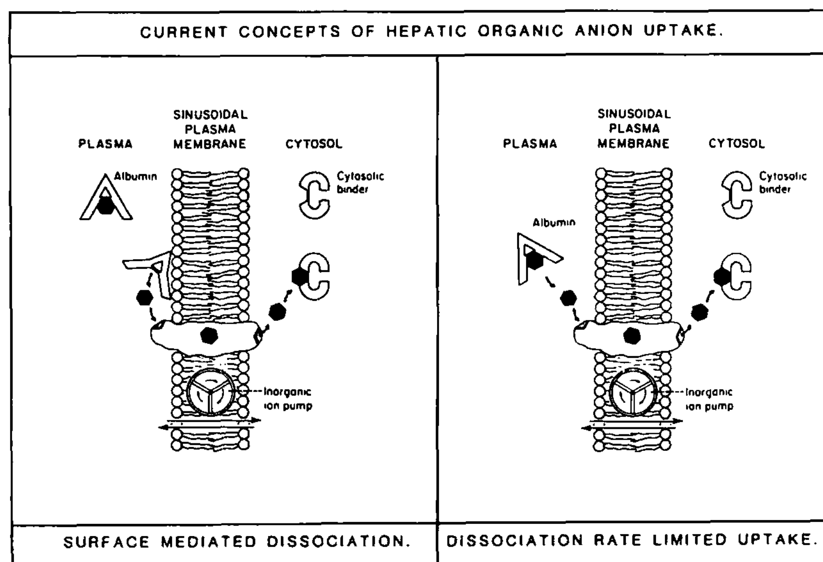

FIGURE 3
Schematic representation of intrahepatic dissociation of anionic drugs from albumin within the hepatic sinusoids and space of Disse. Two mechanisms are indicated: surface interaction of albumin leading to conformational change and facilitation of drug dissociation (left) and facilitative dissociation via progressive removal of the unbound fraction during passage through the liver (right)

Albumin receptors

Whether intrahepatic dissociation is due to a specific interaction with an albumin receptor on sinusoidal domain of the plasma membrane, leading to conformational changes in the molecule, is open to question.[28,29] Until now the presence of specific albumin binding sites on the surface of hepatocytes is still debated[23,29] and uptake kinetics of various albumin-bound substrates certainly do not show an identical K_m related to saturable binding of albumin to the cell surface. Rather aspecific contacts of albumin with the cell surface or membrane–fluid interface may lead to perturbations in the molecule.[30,31] For instance, the kidney also efficiently extracts protein-bound organic compounds from the plasma without having the opportunity to have intimate contact between plasma proteins and the plasma membranes of the tubular cells, since endothelial fenestrae, as they occur in the liver, are not present.[24]

The hypothesis of involvement of a specific albumin receptor was further challenged by the finding that the basic drugs propranolol[32,33] and methyldeptropine,[34] agents that bind to α_1-acid glycoprotein, for which no evident receptor is present on the hepatocyte surface, also show a dissociative effect. Clearance of these drugs by isolated perfused rat livers is virtually unaffected by addition of this protein, even though drug binding in these cases is markedly increased. It should be realized, however, that the binding constants for these drugs to the protein are relatively low and spontaneous dissociation due to progressive removal of the free fraction may also play a role.

It is of interest that recently a promoting effect of albumin on initial uptake rate was observed in isolated hepatocytes for iopanoic acid[35] and in basolateral plasma membrane vesicles for taurocholate,[36] suggesting that some sort of interaction might occur even in such preparations without the presence of flow and acinar gradients. The facilitating effect on vesicle uptake, however, was most prominent at concentrations between 18 and 37 μM, at least ten times lower than the physiological albumin concentration. Nevertheless, uptake in the range of 400 μM albumin was less reduced than anticipated on the basis of the unbound fraction. Iopanoic acid is a very hydrophobic compound and albumin may have played a role in presentation of this poorly water-soluble compound to the surface of the hepatocytes or alternatively may have facilitated passage of unstirred layers in this system. As mentioned before, such an effect of albumin was not observed in the uptake of DBSP in hepatocytes.[10]

One might argue that the use of albumin in the case of hepatocyte and vesicle studies with taurocholate protected plasma membranes *in vitro* from general damaging effects of the bile salts, providing an explanation for the 'promoting' effect of albumin for this compound. However, a beneficial effect of albumin in the unbound clearance of prazosin and antipyrine, drugs that only slightly bind to albumin, was also observed in isolated perfused rat liver.[37] Such an effect was not seen with α_1-acid glycoprotein and may indicate an albumin-specific effect on the functional integrity of the *in vitro* preparations.

Model dependency of clearance calculations

As already proposed by Coburn,[38] the kinetic behaviour in perfused rat livers of taurocholate,[18] other organic anions[28] as well as propranolol[28,32,33] at different protein concentrations can be readily explained by the classical 'venous equilibrium' model or 'well stirred' perfusion model. The assumption of surface-mediated dissociation is only necessary if the 'venous equilibrium' model is rejected and a 'sinusoidal perfusion' or 'parallel tube' model is employed to predict the kinetic behaviour.[28] The latter, evidently more physiological model predicts a more efficient extraction at low values of fu and a relatively larger decrease in hepatic extraction if fu is decreased.

Since extraction of organic anions was experimentally shown to be quite insensitive to addition of albumin, the 'parallel tube' model, but not the 'well stirred' model requires some sort of debinding mechanism to accommodate the data.[25,28] Autoradiographic studies for taurocholate[39] and propranolol[40] in the liver demonstrated a heterogeneous distribution with clear concentration gradients along the axis of the hepatic acinus, clearly invalidating the idea of the liver behaving as a well-stirred compartment. Nevertheless it should be realized that preferential uptake in zone 1 does not necessarily imply that metabolic or excretory clearance is also predominant in that zone. For instance, there is evidence that phase 1 metabolism is more important in the distal zone 3.[27,41] Consequently, the unequal sites of acinar accumulation and clearance processes may for some drugs lead to a situation that is compatible with the 'well stirred' model. Such conditions may be even more adequately modelled in more sophisticated 'dispersed models' as has been recently proposed.[41]

The prediction of changes in clearance and bioavailability with changes in fu is quite different for the proposed models, especially at high extraction ratios.[42-45] For instance, the 'well stirred' model[44] indicates no change in the area under the concentration–time curve of unbound drug (AUCu) after oral administration with a variable fu (increased clearance and decreased bioavailability compensate for the increase in fu). In contrast there is a marked change in the AUCu in the 'parallel tube' model, while the 'dispersed' model takes an intermediate position.[41] Also such models yield different answers calculating clearance *in vivo* from *in vitro* enzyme parameters such as V_{max} and K_m.[28,46-48] Part of the controversy in proving the 'best model',[28,49] for

instance for the hepatic clearance of propranolol,[32 33 42 50] diazepam,[43] taurocholate,[18 28 38] BSP and DBSP,[7 25] may be explained by differences in experimental conditions such as the chosen drug/protein concentration ratio,[25] the influence of perfusion pressure on the distribution of flow in the organ, deviations from first-order kinetics and the unwarranted assumption that the unbound concentration does not change if the substrate and protein concentrations are increased at a constant molar ratio.

At the moment therefore it remains to be established to which extent spontaneous and surface-facilitated dissociation play a role. In conjunction it should be realized that the relative contribution of these mechanisms to the extraction of drugs by the liver may largely depend on the type of compound: hydrophobic ligands with very high affinity (dissociation constant < 10^{-7} *M*) for plasma proteins such as fatty acids, BSP, iopanoate and bilirubin may (partly) require surface interactions to provide sufficient dissociation before associating with the carrier sites. For other drugs with lower affinities, such as BSP-GSH and propranolol, spontaneous dissociation may be the most prominent mechanism. Under certain conditions even dissociation of the substrate from albumin may represent the rate-limiting step[25] and instead of equilibrium binding constants the 'off rate' for dissociation should be determined as the relevant parameter (Fig. 4). Recent studies indicate that lactosylation of albumin does not change the equilibrium binding constant for the organic anion DBSP, but in contrast clearly decreases the 'off-rate' constant. This phenomenon may explain the slower uptake rate of DBSP in isolated perfused rat livers if albumin in the perfusion medium is replaced by the lactosylated protein.[51]

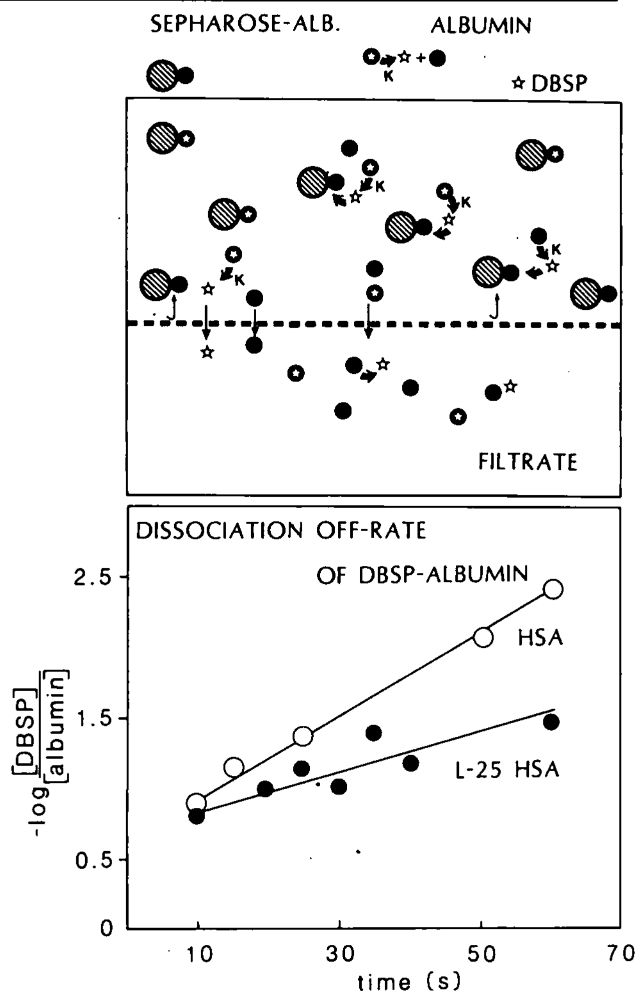

FIGURE 4
Determination of the 'off-rate' dissociation constant in protein binding. Excess of albumin, immobilized by coupling to cepharose, is added, rapidly mixed with a solution of dibromosulfophthalein (DBSP) with normal albumin and subsequently ultrafiltered. Initial binding of DBSP to albumin is > 99%. In time DBSP molecules will dissociate from albumin and become bound to the non-filtrable albumin. Since the dissociation step is rate-limiting, the decline in concentration of filtrable DBSP is a measure for the dissociation 'off-rate' of the DBSP–albumin complex

Clinical implications

Liver disease evidently should not be treated as a homogeneous pathological condition: with regard to effects on protein binding and clearance of drugs chronic diseases such as cirrhosis yield a completely different situation than for example acute viral hepatitis or liver carcinoma.[52] In liver cirrhosis albumin concentration is often lowered, but in addition metabolic functions are decreased. The latter aspect does not only imply that rate of drug metabolism can be decreased, but also that multiple substrates that are no longer efficiently removed by the liver, accumulate in the general circulation. This in turn may affect plasma and tissue binding or even the therapeutic and/or side effects of drugs directly. It follows that not only the nature but also the stage of the pathological condition will determine the final outcome on drug clearance and unbound plasma concentration.

In order to understand the consequences for a particular drug in the individual patient it is necessary to know about the rate-limiting steps in the clearance process, the actual mechanism of the clearance process (oxidation, conjugation, excretion) and to differentiate in immediate effects on unbound plasma concentration as opposed to final changes in that parameter in the steady-state situation.[1 2 5 52-54] Alterations in blood flow, protein binding, intrinsic clearance and tissue binding may have very different effects on the unbound concentration of various classes of drugs. Changes in hepatic clearance by perturbation of protein binding[2 5 42 44] due to disease or drug interaction[53 54] at the level of protein binding therefore may affect elimination rate, but also bioavailability of enterally administered drugs.[5 44] These principles can be illustrated by the three examples mentioned earlier:

clearance of the anionic drug BSP measured for diagnostic reasons, clearance of the antidiabetic drug tolbutamide (both are avidly bound to albumin) and kinetics of the beta-blocking agent propranolol that in plasma is mainly associated with α_1-acid glycoprotein.

LIVER IMPAIRMENT AND BSP

Both initial disappearance rate of BSP, mainly reflecting distribution to the liver, and total clearance calculated from the well-known biexponential disappearance patterns are affected in liver disease to a varied extent,[8] depending on the type and stage of the disease. A decrease in initial disappearance rate will be noticed if hepatic blood flow is lower. With cholestatic conditions and genetic defects in hepatic storage or biliary transport, characteristic changes are seen in the secondary component of the disappearance curves. However, if due to chronic liver disease plasma albumin is abnormally low, *fu* of the dye will be increased and clearance will tend to increase even though this will not linearly follow the relative change in *fu* as discussed above.[7 8] In addition, extrahepatic clearance may increase due to lower protein binding.[7 26] A decreased intrinsic clearance by the diseased hepatocytes can therefore be masked by the opposing effect of decreased protein binding and false-negative results may interfere with the diagnosis of such conditions with the dye. Measurement of the unbound concentration would be anticipated to improve interpretation of the data.[7 11]

LIVER IMPAIRMENT AND TOLBUTAMIDE

The second example is the paradoxically increased clearance of tolbutamide found in certain chronic liver diseases.[52] Since tolbutamide is a 'low-clearance' drug, hepatic clearance is normally linearly related to *fu*. Because the distrubtion volume is quite small, the observed increased in *fu* will only moderately affect this parameter and consequently elimination $t_{1/2}$ will decrease. In contrast, bioavailability will not be influenced to a great extent since hepatic extraction is low. Chronic liver disease with an implicit decreased cellular function in this particular case is 'over-compensated' by decreased protein binding. Since the increase in *fu* is compensated by the lowering of the total steady-state plasma concentration due to increased clearance, unbound concentration will not markedly change and adjustment of the maintenance dose is not necessary. Fluctuations within the dosing interval as determined by the $t_{1/2}$, however, will be increased.

LIVER IMPAIRMENT AND PROPRANOLOL

The third example is propranolol. The unbound fraction is largely determined by the concentration of α_1-acid glycoprotein under various pathological conditions.[1 2] Since the plasma levels of this acute-phase protein increase in conditions such as rheumatic disorders, Crohn's disease, myocardial infarction, tumours *etc.*,[2 3] *fu* will tend to decrease under such conditions. The reverse is true with chronic liver disease, since under that condition less of the protein is synthesized. A decrease in *fu* during

TABLE III
*Protein binding and pharmacokinetics of propranolol**

	Hypoproteinaemia	Acute-phase reactions
Unbound fraction (*fu*)	0.10 → 0.14	0.10 → 0.06
Clearance (ml/min)	910 → 1010	910 → 730
Intrinsic clearance (ml/min)	2600 → 3640	2600 → 1560
Hepatic extraction	0.65 → 0.72	0.65 → 0.52
Bioavailability	0.35 → 0.28	0.35 → 0.48
Distribution volume (ml/70 kg)	280 → 390	280 → 170
Elimination $t_{1/2}$ (min)	215 → 270	215 → 162
Total steady-state concentration after oral administration	21.5 → 15.4	21.5 → 36.5
Steady-state concentration of unbound drug after oral administration	2.15 → 2.15	2.15 → 2.15
Loading dose (mg)	30	
Maintenance dose (mg)	20	
Dose interval (min)	360	
Total steady-state concentration after intravenous administration	61 → 55	61 → 76
Steady-state concentration of unbound drug after intravenous administration	6.1 → 7.7	6.1 → 4.6

* A theoretical example of propranolol pharmacokinetics in the cases of decreased protein binding due to renal protein loss and increased binding due to a rise in α_1-acid glycoprotein after an acute-phase reaction. First-order kinetic conditions, unchanged hepatic intrinsic cellular function and blood flow are assumed (calculations are performed according to the 'well stirred' model with the equations depicted in Table II). In spite of marked changes in distribution volume and clearance, the final steady-state unbound concentration after pertubation of protein binding is unchanged after oral dosing, but in contrast is changed after parenteral administration. The first figure indicates the 'normal' situation, the second figure the situation with changed protein binding.

acute-phase reactions will decrease hepatic (blood flow-limited) clearance of propranolol moderately. However, the distribution volume of this beta-blocker is large and according to equation 3 (Table II) will almost linearly decrease with *fu*, so that as a result of both changes $t_{1/2}$ will be shortened. Hepatic extraction, which is normally high, will tend to decrease and bioavailability will therefore tend to increase as has been demonstrated in patients with Crohn's disease.[1] Since bioavailability increases, but at the same time clearance decreases less, average total drug concentrations in steady-state will be higher, while fluctation in steady-state will be considerably larger due to the decreased distribution volume. If we assume that the 'well stirred' model adequately describes the hepatic clearance of propranolol,[32,33] it is of interest that at changes in *fu* the mean unbound concentrations of propranolol (*Cu*) should only change after parenteral, but not with oral administration (see Table III). Dose adjustment is only necessary with intravenous dosing and should include an increased maintenance dose that is practically more feasible than a decreased dose interval.

Conclusion

In conclusion it should be stressed that in carefully selected cases of drug monitoring[11] as well as in the diagnosis of liver disease with transport model compounds, one could largely benefit from determination of unbound concentrations instead of total plasma concentrations of the particular compounds. It should be realized that alteration in plasma protein binding due to disease or interactions with other drugs cannot be simply translated in changes in the unbound plasma concentration in steady-state, since many kinetic parameters, such as clearance, distribution volume and bioavailability, may change at the same time in different directions and to a different extent. This may depend on the extent of binding in plasma and tissues, the nature of the binding proteins as well as the rate-limiting steps in body clearance. It should be emphasized also that changes in plasma protein levels are rarely a separate entity and are often accompanied by changes in intrinsic renal or hepatic function and/or accumulation of endogenous substrates than can interfere with elimination and distribution of the drug.

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