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EFFECT OF PROTEIN BINDING ON THE PHARMACOLOGIC ACTIVITY OF DRUGS AS EXEMPLIFIED BY WARFARIN

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INTRODUCTION

Many drugs interact with proteins to form reversible drug-protein complexes. Such complexes have different properties from the free (unbound) drug and, consequently, protein binding can influence both the pharmacokinetic and pharmacodynamic characteristics of drugs. It is relatively easy to obtain blood from different normal subjects and from many types of patients for plasma or serum protein binding studies, particularly if the drugs are added to the plasma in vitro. This ease of procurement may be partly responsible for the large literature on the protein binding of drugs in plasma. Conversely, the technical difficulties associated with determinations of drug binding to tissues are reflected by the very limited amount of published information on that subject. The relative amounts of available knowledge and information concerning the plasma protein and tissue binding of drugs are actually quite out of proportion to the relative importance of these two types of processes. To the extent that reversible interactions with endogenous macromolecules affect the distribution and time course of drug concentrations in the body, these effects are due to interactions that occur both in plasma and tissues, and often reflect a balance between the forces that tend to localize the drug at one or the other of these sites.

Most systemic pharmacologic effects of drugs arise from interactions with certain receptors or end-organs and are concentration-dependent. Similarly, drug biotransformation processes usually

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involve an interaction with certain enzymes and proceed at rates that are a function of the drug concentration. In more precise terminology, the intensity of systemic pharmacologic effects and the rates of drug biotransformation are functions of the concentration of that form of drug which is accessible to, and capable of interacting with the relevant receptors or enzymes. Specifically, what counts is the concentration of free drug in the immediate environment of these specific sites of interaction—i.e., in the appropriate biophase. To the extent that protein binding to other types of sites—particularly proteins in plasma and tissues—can alter the magnitude and time course of free drug concentrations in the biophase, these nonspecific binding processes can affect quantitatively the magnitude and time course of both pharmacologic activity and drug elimination.

The coumarin anticoagulant drug warfarin is particularly suitable for the exploration and illustration of the potential effects of plasma protein and tissue binding on the pharmacokinetics and pharmacodynamics of drugs. Sensitive and specific assays are available to determine the concentrations of warfarin in plasma and tissues, radiolabeled forms of the drug can be obtained commercially, and—this being particularly important—the pharmacologic effect of warfarin can be measured objectively and precisely. To describe and discuss the pharmacodynamic implications of drug-protein binding in this overview, we will begin by considering the plasma protein binding of warfarin and its pharmacokinetic implications and then turn to the effect of plasma protein binding on the pharmacodynamics of this anticoagulant drug. We will continue by considering the tissue uptake of warfarin and its pharmacokinetic implications. Finally, the effect of tissue uptake on the pharmacodynamics of warfarin will be examined. It will be important for the reader to keep in mind that the general characteristics displayed by warfarin in response, or in relation, to different or altered binding conditions are not unique to that drug but rather are illustrative of the behavior of most other drugs, particularly those whose elimination kinetics are not limited or affected by the rate of blood flow through organs of elimination—i.e., sites of biotransformation and/or excretion.

PLASMA PROTEIN BINDING* AND PHARMACOKINETICS OF WARFARIN

Warfarin is very extensively bound to albumin in plasma. At usual therapeutic plasma concentrations (~ 1 mg/liter), the drug is

*No distinction will be made in this article between plasma and serum.

more than 97 percent, and often more than 99 percent, bound in human and rat plasma (Yacobi, Lampman, and Levy 1977; Slattery, Yacobi, and Levy 1976). The warfarin free fraction—i.e., the ratio of concentrations of free to the sum of free and bound drug—in plasma is independent of drug concentration over a very wide range (Yacobi and Levy 1975a; Yacobi, Udall, and Levy 1976a). There are large and reproducible interindividual differences in warfarin plasma protein binding among normal human subjects and patients (Yacobi, Udall, and Levy 1976a,b and Yacobi, Lampman, and Levy 1977), and particularly among rats (Slattery, Yacobi, and Levy 1976; Yacobi and Levy 1977). The warfarin free fraction values varied about four-fold among 57 human volunteers and about fifteen-fold in a group of 209 male Sprague-Dawley rats. The frequency distribution of these values was log-unimodal in the human subjects and trimodal in the rats.

Theoretical considerations indicate that, for a drug such as warfarin which is eliminated by hepatic biotransformation and whose total clearance is very much lower than the hepatic blood perfusion rate, the total plasma clearance (TC) is equal to the product of the drug's free fraction in plasma (f) and the intrinsic clearance (k'') (Levy and Yacobi 1974):

$$TC = fk'' \qquad \text{(Equation 1)}.$$

The intrinsic clearance of the drug reflects the sum of the activities of the enzyme systems that act on warfarin and convert it to its several metabolites. Compared to the pronounced interindividual variation of warfarin free fraction (protein binding) in plasma, the variation of intrinsic clearance values is relatively small, particularly in rats but also in humans. Consequently, one can readily demonstrate a linear relationship between the total plasma clearance of warfarin and the plasma free fraction of warfarin (Yacobi and Levy 1977; Yacobi, Udall, and Levy 1976a). As shown for rats in Figure 22-1, an order of magnitude range of interindividual differences in total clearance of warfarin is almost entirely due to corresponding differences in the plasma protein binding (free fraction) of the drug.

For drugs with linear pharmacokinetic characteristics, the steady-state plasma concentration (C_{SS}) is a function of dosing rate (R) and total clearance:

$$C_{ss} = R/TC \qquad \text{(Equation 2)}.$$

Combination of Equations 1 and 2 and rearrangement yields

$$fC_{ss} = R/k'' \qquad \text{(Equation 3)}.$$

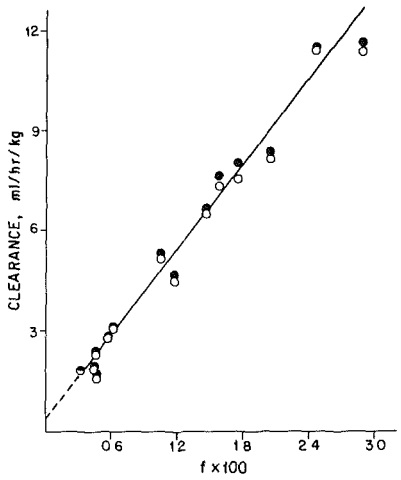

FIGURE 22-1
Relationship between total clearance and serum free fraction (f) of warfarin in rats. Total clearance was calculated by two methods, designated by solid and open circles, respectively.
Source: Yacobi and Levy (1977). Reproduced with permission of copyright owner.

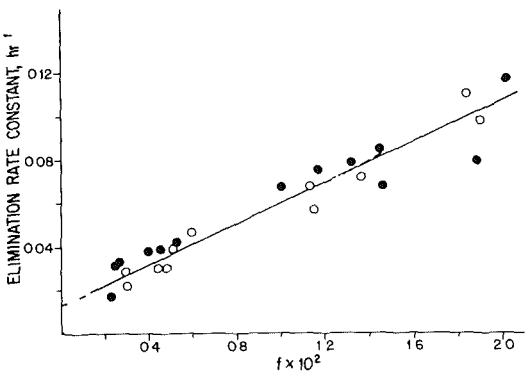

FIGURE 22-2
Relationship between the elimination rate constant and the serum free fraction of warfarin in rats. Results of two sets of experiments, designated by solid and open circles, respectively.
Source: Yacobi and Levy (1975a). Reproduced with permission of copyright owner.

Thus, according to Equation 3, the steady-state plasma concentration of free drug is independent of plasma protein binding and if, as is commonly held, the pharmacologic activity of a drug is a function of the concentration of free rather than total drug, then differences in plasma protein binding (all else being equal), should have no effect on the drug's pharmacologic activity at steady state. That reasoning is correct when the drug is administered continuously at a constant rate (i.e., by intravenous infusion or by certain other types of controlled delivery systems), but it does not apply when drug administration is intermittent (i.e., by tablets or repeated injections).

The total clearance of a drug with linear pharmacokinetic characteristics can be separated into two components, the apparent volume of distribution (V) and the elimination rate constant (k):*

$$TC = Vk \qquad \text{(Equation 4)}.$$

The volume of distribution depends not only on plasma protein binding but also on tissue binding and on other forms of tissue localization. In the case of warfarin, large interindividual differences in plasma protein binding (f) and therefore in total clearance are associated with relatively small differences in V (Yacobi and Levy 1975a). Consequently, changes in f produce large changes of k and therefore of the biologic half-life ($t_{\frac{1}{2}}$), since $k = 0.693/t_{\frac{1}{2}}$. An example of the relationship between k and f of warfarin in rats is shown in Figure 22-2.

PLASMA PROTEIN BINDING AND PHARMACODYNAMICS OF WARFARIN

Warfarin inhibits the synthesis of vitamin K-dependent clotting factors in the liver and thereby acts as an indirect anticoagulant. Clinically, this anticoagulant effect is reflected by the prothrombin time of plasma which in turn can be converted to prothrombin complex activity or PCA (Wingard and Levy 1973). For practical purposes, the kinetics of PCA can be characterized in terms of synthesis

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*The use of k is appropriate for drugs that confer upon the body the pharmacokinetic characteristics of a one-compartment system. In other cases, k is replaced by β, where $-\beta/2.303$ is the slope of the terminal exponential phase of a plot of log concentration versus time and intravenous injection of the drug.

rate (R_{syn}) and elimination rate constant, where warfarin acts on the synthesis process (Nagashima, O'Reilly, and Levy 1969). Both in humans and rats there exists an apparently linear relationship between the degree of inhibition of PCA synthesis and the logarithm of the warfarin concentration in plasma (Nagashima, O'Reilly, and Levy 1969; Yacobi, Wingard, and Levy 1974). It has been customary to plot R_{syn}/R^0_{syn} versus log warfarin concentration, where R^0_{syn} is the baseline synthesis rate of PCA. This type of plot typically shows a negative linear relationship between the variables. Figure 22-3 provides examples of this relationship for the two enantiomers of warfarin, which differ in anticoagulant potency (Yacobi and Levy 1974).

There are large interindividual differences in the warfarin plasma concentration-anticoagulant response relationship that are not ascribable to corresponding differences in end-organ sensitivity to the drug but that are due to variable plasma protein binding. For example, in a group of 12 rats that received a dose of S(-)-warfarin, 0.6 mg/kg, the plasma concentration of total drug required to obtain 50 percent inhibition of normal PCA synthesis rate (i.e., $R_{syn}/R^0_{syn} = 0.5$) ranged from 0.155 to 1.28 mcg/ml and the coefficient of variation was 75 percent of the mean. When these concentrations were expressed in terms of free drug, the range narrowed from 0.00166 to 0.00401 mcg/ml and the coefficient of variation was reduced to only 24 percent of the mean (Yacobi and Levy 1975b). The individual results of this study are shown in Figure 22-4. They indicate that free rather than plasma protein bound warfarin is the pharmacologically active form of the drug.

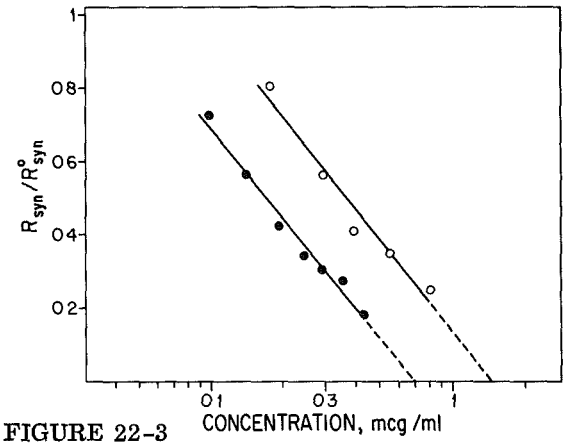

FIGURE 22-3
Relationship between relative synthesis rate of prothrombin complex activity (R_{syn}/R^0_{syn}) and the plasma concentration of R(+)-warfarin (open circles) or S(-)-warfarin (solid circles) in a rat.
Source: Yacobi and Levy (1974). Reprinted with permission.

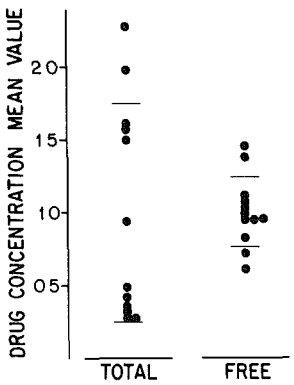

FIGURE 22-4
Relative plasma concentrations of total and free S(-)-warfarin required to decrease the synthesis rate of prothrombin complex activity to one-half of normal in 12 rats. The horizontal bars indicate ± one relative standard deviation.
Source: Yacobi and Levy (1975b). Reprinted with permission of PJD Publications Limited, P.O. Box 966, Westbury, N.Y. 11590.

Considering now the pharmacokinetic and pharmacodynamic aspects of warfarin binding to plasma proteins together, it is evident that changes or interindividual differences in warfarin binding to plasma proteins affect the anticoagulant activity by modifying the time course of free warfarin concentrations in plasma during the clinically usual intermittent (typically, once daily) administration of the drug and, of course, after administration of a single dose. If, for example, the free fraction is increased due to a substantial decrease of the plasma albumin concentration or due to administration of an effective competitive binding inhibitor (and assuming that there are no other effects such as inhibition of enzyme systems or direct perturbation of the blood clotting system), then there will occur a decrease in the biologic half-life and more pronounced excursions of plasma warfarin concentrations during a dosing interval at steady state. Specifically, the plasma concentrations of free warfarin will have higher maxima and lower minima even though the time-averaged concentration during a dosing interval at steady state will remain the same. Consequently, there will be an increase of the maximum effect and a decrease of the minimum effect during the dosing interval (Levy 1976). The magnitude of these changes is relatively modest for warfarin (Wingard and Levy 1977) but can be pronounced for drugs with relatively steep concentration-pharmacologic activity relationships especially when such drugs are administered in rapidly absorbed form and at intervals that are long relative to their half-life (Levy 1976). The duration of effect of single doses of warfarin and other

drugs with similar characteristics may be increased or decreased by decreased plasma protein binding, depending on the dose and the pharmacologic endpoint (Levy 1976).

TISSUE BINDING* AND PHARMACOKINETICS OF WARFARIN

The concentrations of warfarin can be substantially higher in certain tissues, notably the liver, than in plasma. This depends very much on the concentration of the drug, as is evident from the results of a study (Takada and Levy 1979), some results of which are summarized in Table 22.1. The liver-to-serum concentration ratio of warfarin six hours after an i.v. injection of the drug increased almost fourteen-fold on the average when the dose was reduced from 1.0 to 0.1 mg/kg. This difference is not due to a change in plasma protein binding since the plasma free fraction of warfarin is independent of concentration in this range. Inferring from these results that warfarin binding to hepatic tissue is concentration dependent, and therefore that the apparent volume of distribution of the drug is concentration-dependent, one would expect that postdistributive plasma warfarin concentrations do not decline exponentially with time (Gibaldi, Levy, and McNamara 1978; McNamara et al. 1979). In fact, they apparently do (Figure 22-5), but there is another type of change: the apparent volume of distribution is appreciably decreased as the dose of injected drug is increased (Takada and Levy 1980).

A more detailed examination of the concentration dependence of the distribution of warfarin between the tissues and plasma of rats provides an explanation for the unusual dose dependence in the pharmacokinetics of warfarin. Calculations based on data from our laboratory and those of others yielded the distribution profile shown in Figure 22-6; this has been confirmed and extended by detailed laboratory investigations with the S(-) enantiomer of warfarin (Cheung and Levy 1985a). The type of distribution profile shown in Figure 22-6 was found to be independent of dose, time, and even plasma free fraction of warfarin. Similar profiles were obtained for tissues other than the liver. These profiles are consistent with the presence of two classes of reversible binding sites in the tissues, one being of high affinity and low capacity for warfarin, the other being of low

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*No distinction will be made in this discussion between tissue binding and other concentrative uptake mechanisms including partitioning and specialized transport of the drug through biologic barriers.

TABLE 22.1
Effect of Dose on the Distribution of Warfarin in Rats

Results Obtained 6 Hours After I.V. Injection	0.1 mg/kg	1.0 mg/kg
Serum conc., mcg/ml	0.102 ± 0.010	4.65 ± 1.14
Liver conc., mcg/g	1.14 ± 0.06	3.58 ± 0.34
Serum free fraction × 100	1.30 ± 0.15	0.830 ± 0.366
Liver-to-serum total conc. ratio	11.3 ± 1.7	0.814 ± 0.222
Liver total-to-serum free conc. ratio	866 ± 105	111 ± 42

Note:
Results are expressed as mean ± S.D., n = 6.
Source:
From Takada and Levy (1979). Reproduced with permission of copyright owner.

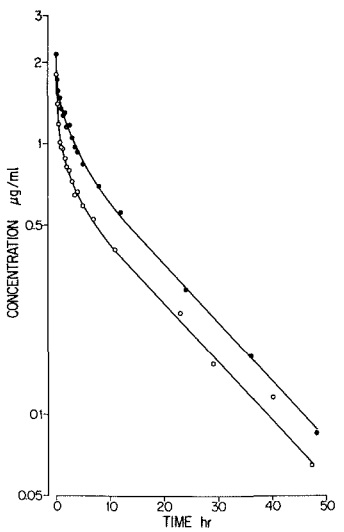

FIGURE 22-5
Effect of drug concentration on the pharmacokinetics of warfarin in a rat that had received an intravenous injection of a tracer dose of ^{14}C-warfarin 12 hr after injection of saline solution (control experiment, open circles) and, 1 week later, the same dose of ^{14}C-warfarin 12 hr after intravenous injection of nonradioactive warfarin, 0.5 mg/kg (solid circles). The data points show the time course of ^{14}C-warfarin concentrations in serum. The apparent volume of distribution was significantly decreased in the second experiment.
Source: Takada and Levy (1980). Reproduced with permission of copyright owner.

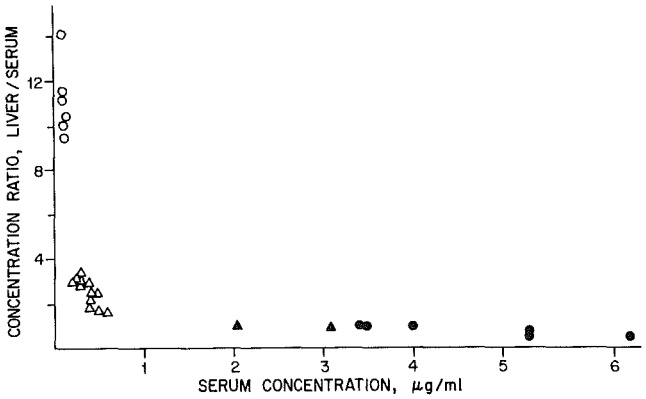

FIGURE 22-6
Liver-to-serum total concentration ratio of warfarin as a function of the serum concentration in rats that had received a single dose of racemic warfarin. Based on data from various published reports. Source: Cheung (1984).

affinity and apparently unlimited capacity. A suitable mathematical expression of this situation is:

$$C_T = \frac{T_{max} f(C_p)}{K + f(C_p)} + P(C_p) \qquad \text{(Equation 5)}$$

where C_T and C_P are the total drug concentrations in the tissue and plasma, respectively, T_{max} is the maximum capacity of the high affinity binding sites and K is the dissociation constant of the complex formed between these sites and warfarin, f is the plasma free fraction of the drug, and P is the proportionality constant for the distribution of the drug between the low affinity-unlimited capacity binding sites and the plasma.

In the case of warfarin, K is much smaller than the lowest $f(C_P)$ value usually measured in clinical or experimental studies so that Equation 5 can be simplified to:

$$C_T = T_{max} + P(C_p) \qquad \text{(Equation 6)}$$

which, upon rearrangement, takes the form:

$$C_T/C_p = (T_{max}/C_p) + P \qquad \text{(Equation 7)}$$

Equation 7, in which C_T/C_P is the tissue/plasma concentration ratio of the drug, is the most useful way of characterizing tissue uptake because this ratio is used directly in physiologic-pharmacokinetic modeling (Gibaldi and Perrier 1982). Our studies have shown that both T_{max} and P for warfarin are independent of f with the exception of fat where P is independent of fC_P (Cheung and Levy 1985a).

In practical pharmacokinetic terms, these findings mean that a certain absolute amount of warfarin is very tightly sequestered by the tissues, so much so that it almost disappears pharmacokinetically. Thus, any dose D administered to a warfarin-free animal is reduced promptly by an amount equal to ΣT_{max}—i.e., by the sum of the T_{max} values for the individual tissues, while the remainder (i.e., $D - \Sigma T_{max}$) is subject to linear pharmacokinetics. As a consequence, plasma warfarin concentrations decline apparently exponentially but the apparent volume of distribution calculated from the plasma concentrations and the dose appears to decrease with increasing dose as evident in Figure 22-5. The situation is, however, more complicated. When plasma warfarin concentrations decrease to very low values, much lower than one would ordinarily wish to measure, and thereby approach the value of K, then the drug begins to be eliminated from its high affinity binding sites in the tissues. It can be shown analyt-

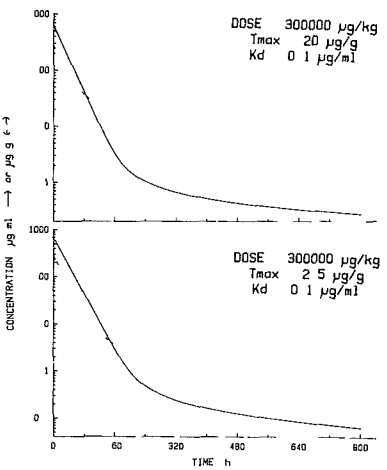

FIGURE 22-7
Simulated time course of concentrations in blood (continuous line) and tissues (stippled lines) of a hypothetical drug whose free fraction in blood is independent of concentration and whose binding to tissues involves a high affinity and low capacity process as well as a low affinity and unlimited capacity process.
Source: Cheung (1984).

ically and by computer simulation (Cheung 1984) that, under these conditions, the apparent half-life of warfarin will increase drastically. An example which demonstrates this in principle is shown in Figure 22-7. In the case of warfarin, the difference in half-life at ordinary and very low plasma concentrations exceeds one order of magnitude.

TISSUE BINDING AND PHARMACODYNAMICS OF WARFARIN

Due to the very high affinity binding of warfarin to the liver, muscles, and other tissues, significant amounts of the drug can be demonstrated at these sites several months (!) after administration of a single dose (Cheung and Levy 1985b). The persistence of the drug in the liver is particularly noteworthy, for this is the organ in which warfarin produces its pharmacologic activity. Nevertheless, the time course of the anticoagulant effect of warfarin is a function of the drug's concentration-time pattern in plasma and not in the liver. The reason for this is that most of the drug in the liver is bound to non-specific binding sites and thus is not "seen" by the pharmacologic target sites. The target sites, and more directly the biophase in which these sites are located, are in rapid equilibrium with free drug in plasma and it is these concentrations that are "seen." Moreover, since nonspecific binding to hepatic tissues is nonlinear, total warfarin concentrations in the liver are not directly proportional to the concentrations of free drug.

There are additional, important considerations. The tightly bound warfarin in tissues can be readily mobilized. If, for example, one administers a small dose of radiolabeled warfarin to rats and, ten days later, follows with a relatively large dose of nonradioactive drug, then one finds a sudden and appreciable rise of radioactivity in the plasma (Cheung and Levy 1985b). That radioactivity is warfarin as determined by both thin layer chromatography and high pressure liquid chromatography. Concomitantly, warfarin concentrations in the tissues decrease drastically. Presumably, and this is being studied at present, certain other drugs are also capable of displacing warfarin from the tissues.

A preliminary review of plasma and tissue concentration data for other drugs suggests that several of them may exhibit the same type of tissue binding characteristics as does warfarin. It may not be rare to find drugs that persist at high affinity tissue binding sites for months after the last dose has been given. For example, morphine has been found in the tissues of rats 22 days after abrupt withdrawal of the drug (Jones et al. 1984). If drugs can persist in tissues for

weeks or months due to reversible but very high affinity binding, then it is possible that the bound drugs may be suddenly mobilized by the competitive displacing effects of other drugs or of endogenous substances that accumulate when the body's physiologic status is perturbed. This may be particularly troublesome if the capacity of the high affinity binding sites is high relative to the usual therapeutic dose of the drug. If the biologic half-life of the displacing agent is short relative to that of the displaced drug, then the latter may return to its tissue binding sites after the displacing agent has been eliminated from the body. This could, presumably, happen repeatedly. Drugs at high affinity binding sites may therefore be likened to a time bomb ready to go off unexpectedly, and capable in principle of producing delayed pharmacologic effects such as the flashback associated with lysergic acid diethylamide. Thus, it is evident that reversible binding of drugs to high affinity sites in tissues can have very important pharmacokinetic and pharmacodynamic implications. More emphasis should be placed in future research on the reversible interactions of drugs with tissue rather than plasma proteins.

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