Numerical Solution of Nonlinear Pharmacokinetic Equations: Effects of Plasma Protein Binding on Drug Distribution and Elimination

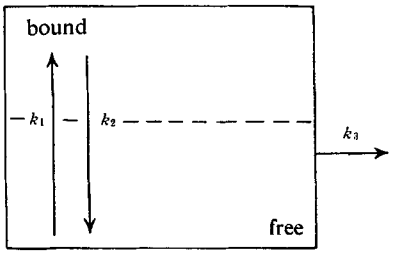
JOHN J. COFFEY, FRANCIS J. BULLOCK, and PAUL T. SCHOENEMANN

Abstract □ Numerical methods are described for the solution of the differential equations arising from nonlinear binding of drugs to plasma proteins, assuming one- and two-compartment pharmacokinetic models. These numerical methods should be of general utility in studying multicompartment models. The application of these methods to several systems, both hypothetical and real, suggested that binding of drugs to plasma protein should cause detectable nonlinearity in the log *C versus t* plot only if doses are sufficiently high to approach saturation of binding sites or if the number of binding sites in plasma is small. The effect of competition for binding sites in plasma on drug concentrations in tissues was studied by assuming a two-compartment model. It appears that, unless the tissue distribution volume is quite small, competition for binding sites would not be expected to have a large effect.

Keyphrases □ Pharmacokinetics—quantitation of nonlinear equations, effect of plasma protein binding on drug distribution and elimination □ Drug–plasma protein binding—quantitation of nonlinear equations □ Binding site competition—drug–plasma, two-compartment model

It is commonly accepted that binding of drugs to plasma proteins plays an important role in the pharmacokinetics and pharmacology of tightly bound substances. Classes of compounds for which protein binding is considered significant include the penicillins (1), sulfonamides (2), anticoagulants (3, 4), nonsteroid anti-inflammatory agents (5), several compounds of interest in cancer chemotherapy (6, 7), and certain endogenous hormones such as thyroxine (8) and the corticosteroids (9). In particular, a great deal of emphasis has been laid on the possibility of adverse drug reactions arising from competition for plasma protein binding sites. Brodie (10) called attention to several cases of drug toxicity where changes in binding may be implicated. Among these, the interactions of warfarin and several sulfonamides with phenylbutazone (3, 4, 11) and the observation of sulfonamide-related kernicterus in the newborn (12–15) are particularly noteworthy.

Despite the long-standing interest in this problem, quantitative consideration of the implications of protein binding in pharmacokinetics has been limited. The lack of closed solutions to the nonlinear differential equations generated by a rigorous statement of the problem has led workers in the field either to adopt highly limited simplifying assumptions or to pursue indirect solutions. The most obvious simplification is to assume a linear relationship between bound and free drug. However, some of the most interesting cases involve drug levels that approach or exceed the binding capacity of the plasma proteins, where such a relationship will not hold. Krüger-Thiemer *et al.* (16) presented some more sophisticated derivations of expressions relating plasma binding to pharmacokinetics, but they found it necessary to assume either a one-compartment model or instantaneous distribution of drug between two compartments. Even in the simple two-compartment case, the solution was presented in an inverse form which does not allow calculation of drug concentrations at specified times. Martin (17, 18) discussed some implications of plasma binding equilibria on drug distribution but he did not treat the kinetic problems in detail.



Scheme I

This article reports a method of attack on the problem which employs approximate numerical integration methods to generate solutions to nonlinear pharmacokinetic equations. In principle, the method outlined for the two-compartment case should be applicable to any multicompartment pharmacokinetic model.

The format adopted for presentation of the results and conclusions is as follows. First, the theoretical basis and assumptions used to calculate concentration–time curves, assuming one- and two-compartment models with binding, are presented. Next, the behavior of the one-compartment model with binding is explored, using a hypothetical drug with certain defined properties. This model is also used to study the pharmacokinetic properties that might be expected if the drug is bound to an easily saturable specific carrier protein. Finally, the behavior of the two-compartment model with binding is explored. With this model, the problem of potential changes in pharmacokinetic and tissue levels resulting from displacement of drug from plasma binding sites is studied using data from the literature.

THEORETICAL

The fundamental assumptions used in this treatment are:

1. Binding occurs only to plasma proteins and follows simple saturation kinetics. The binding process can be described by a single macroscopic dissociation constant.
2. Binding equilibrium is achieved virtually instantaneously with respect to distribution and elimination.
3. Aside from binding, all other processes (*i.e.*, distribution and elimination) are linear.
4. Distribution and elimination processes operate only on free drug.

One-Compartment Model—In Scheme I, let C_b = concentration of bound drug, C_f = concentration of free drug, C_t = total con-

centration of drug, P = total concentration (free plus bound) of protein binding sites, and $K_d = k_2/k_1$, *i.e.*, the dissociation constant of the drug–protein complex.

Since binding follows simple saturation kinetics, the concentration of bound drug can be described by Eq. 16 of Klotz (19). In the notation above:

$$\frac{C_b}{P} = \frac{(1/K_d)C_f}{1 + (1/K_d)C_f} \qquad \text{(Eq. 1)}$$

By rearranging terms:

$$C_b = \frac{PC_f}{K_d + C_f} = C_t - C_f \qquad \text{(Eq. 2)}$$

If $C_f \ll K_d$, Eq. 2 reduces approximately to:

$$C_b = \frac{PC_f}{K_d} \qquad \text{(Eq. 3)}$$

Thus, at sufficiently low values of C_f, there is approximately a linear relationship between bound and free drug. Rearrangement of Eq. 2 yields a quadratic expression in C_f, whose positive root is:

$$C_f = {}^1/_2[-(P + K_d - C_t) + \sqrt{(P + K_d - C_t)^2 + 4K_dC_t}] \qquad \text{(Eq. 4)}$$

Since transfer processes operate only on free drug:

$$\frac{dC_t}{dt} = -k_3C_f = -\frac{k_3}{2} \times [-(P + K_d - C_t) + \sqrt{(P + K_d - C_t)^2 + 4K_dC_t}] \qquad \text{(Eq. 5)}$$

While no closed solution of Eq. 5 is obtained, numerical solutions are simple and convenient. Given a zero-time value of C_t and a time interval, τ, the fourth-order Runge-Kutta approximation is used (20) to calculate a value of C_t at $t = \tau$. The procedure is then repeated starting with this calculated C_t to obtain a value at $t = 2\tau$; in this way, an entire concentration–time curve may be constructed. At each time point, Eq. 4 may be used to calculate C_f from C_t.

Two-Compartment Model—The terms in Scheme II are as already defined, except that V_1 and V_2 are the distribution volumes of the inner and outer compartments, respectively. C_2 is the drug concentration (presumed all free) in the outer compartment, and C_t, C_f, and C_b refer to the inner compartment. If $X_i (i = 2, f, t \ldots)$ is the total amount of drug in a given state and, therefore, $C_i = X_i/V_1$ or V_2, one may write:

$$\frac{dX_t}{dt} = k_{21}X_2 - k_{12}X_f - k_3X_f \qquad \text{(Eq. 6)}$$

$$\frac{dX_2}{dt} = k_{12}X_f - k_{21}X_2 \qquad \text{(Eq. 7)}$$

With the further simplifying assumption (21) that:

$$k_{21} = \frac{V_1}{V_2}k_{12} \qquad \text{(Eq. 8)}$$

the concentration changes in the inner and outer compartments are then given by:

$$\frac{dC_t}{dt} = k_{12}(C_2 - C_f) - k_3C_f \qquad \text{(Eq. 9)}$$

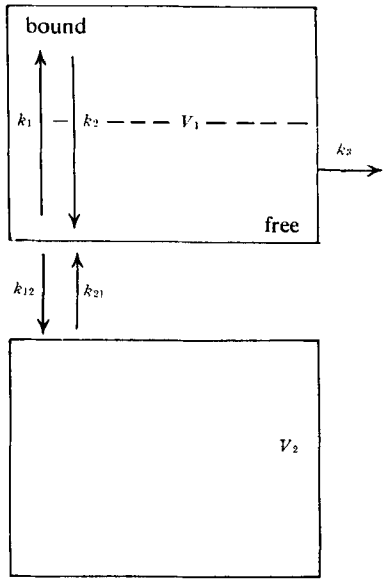
$$\frac{dC_2}{dt} = k_{12}\frac{V_1}{V_2}(C_f - C_2) \qquad \text{(Eq. 10)}$$

Equations 4, 9, and 10 in the three unknowns, C_t, C_f, and C_2, do not yield a closed solution but, once again, numerical methods are available. A solution by successive approximations was chosen, making use of the relationships:

$$\frac{dC_f}{dt} = \frac{dC_t}{dt}\left(1 + \frac{C_t - P + K_d}{\sqrt{(P + K_d - C_t)^2 + 4K_dC_t}}\right) \qquad \text{(Eq. 11)}$$

$$\frac{d^2C_t}{dt^2} = k_1\left(\frac{dC_2}{dt} - \frac{dC_f}{dt}\right) - k_2\frac{dC_f}{dt} \qquad \text{(Eq. 12)}$$

$$\frac{d^2C_2}{dt^2} = k_1\frac{V_1}{V_2}\left(\frac{dC_f}{dt} - \frac{dC_2}{dt}\right) \qquad \text{(Eq. 13)}$$



Scheme II

To start the solution, the first and second derivatives of the three unknowns are calculated at $t = 0$, using Eqs. 9–13 and the initial values $C_t(0) = C_0$ and $C_2(0) = 0$, where C_0 is the administered dose divided by V_1. Taking a time interval τ, C_t and C_2 are estimated at $t = \tau$ by:

$$C(\tau) = C(0) + \tau\frac{dc}{dt} + \frac{\tau^2}{2}\frac{d^2C}{dt^2} \qquad \text{(Eq. 14)}$$

and, similarly, at $t = \tau/2$. These initial estimates are refined by the method of successive approximations (20), evaluating the integrals by Simpson's rule. The final values at $t = \tau$ are then used to obtain values at $t = 2\tau$ by the application of Simpson's rule alone, and the changes of C_t, C_f, and C_2 with time are thus constructed point by point.

FORTRAN programs were written for the Honeywell time-sharing computer system to calculate concentration–time curves, assuming either a one- or two-compartment model, from input values for the model parameters. Several convenient features were incorporated into the programs, notably the capability of handling pharmacokinetic data expressed on a weight basis along with binding data expressed on a molar basis, and a provision for altering the binding parameters at any specified time along the curve to mimic the effect of competition for binding sites.

The one-compartment calculations are quite straightforward, but the two-compartment calculations involve considerable difficulty, chiefly because calculated concentration values tend to oscillate, instead of behaving monotonically, with time. Even with small values of τ and stringent criteria of convergence (currently, 1 part in 10^6 is being used), calculated concentration values begin to oscillate with time after a comparatively short distance along the concentration curve. More sophisticated methods of numerical integration probably would minimize this tendency but would require considerably more computer time.

RESULTS AND CONCLUSIONS FOR A ONE-COMPARTMENT MODEL WITH BINDING

To generate theoretical curves for the one-compartment model, the case of a hypothetical drug with the following properties was considered first:

1. The drug has a molecular weight of 150.
2. Drug binds only to serum albumin, whose concentration is 4.4% and whose molecular weight is 67,000.
3. At sufficiently low drug concentrations, the drug is 89% bound.
4. The distribution volume is 50 ml./kg.
5. Free drug has a half-life of 30 min.

Concentration–time curves for a wide range of drug doses were

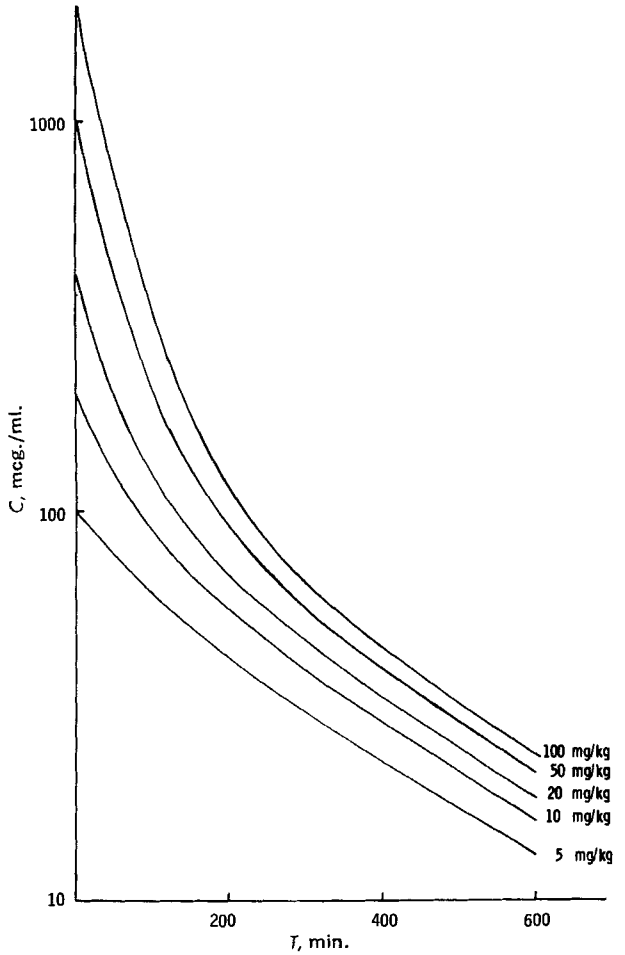


Figure 1—*Calculated concentration–time curves in plasma of a hypothetical drug of mol. wt. 150, 89% bound to serum albumin at low concentrations, with one binding site per albumin molecule. Doses are shown with each curve, and other assumptions are given in the text.*

calculated assuming one binding site (Fig. 1) or 10 binding sites (Fig. 2) per molecule of serum albumin.

At sufficiently low doses, the relationship between bound and free drug is linear and there is an apparent first-order elimination of drug. The rate constant of this elimination process is simply the rate constant for free drug multiplied by the ratio of free drug to total drug:

$$k_{app.} = k_3 \frac{C_f}{C_t} \qquad \text{(Eq. 15)}$$

Regardless of the size of the initial dose, the concentration–time curve eventually approaches this simple exponential decay as the drug concentration falls. However, at high initial doses, there is a phase of more rapid elimination due to the presence of drug beyond the binding capacity of the serum albumin.

With the present assumptions, it appears that substantial curvature of log concentration–time plots is seen only if initial doses are large or if the number of binding sites on the protein is small, permitting drug to exceed the binding capacity of the protein. It seems reasonable to expect that blood levels of many drugs will fall in the region where the binding relationship is linear (Eq. 3), at least in the absence of competitive binding by another species.

It is not uncommon to interpret curved log concentration–time plots as indicative of a multicompartment distribution of drug. While the equations derived when binding is not linear are not multiexponential in form, it is of interest to determine how closely the calculated curves for such a situation can be fitted by a multiexponential function. Figure 3 shows the result of an attempt to fit the 100-mg./kg. curve of Fig. 2 to the expression derived by assuming a two-compartment model without binding, using an unweighted least-squares method similar to that of Wagner and Metzler (22). The individual points are those from which the curve of Fig. 2 was constructed, calculated by the nonlinear binding program, while the smooth curve is the least-squares fit to the biexponential, with the kinetic parameters as shown. In an experimental situation, the noise inherent in the data would most likely mask the small systematic deviation, giving the appearance of an even closer fit. Several experiments of this type revealed no simple relationship between the binding parameters used to generate the points of Fig. 3 and the parameters calculated from a least-squares fit to the standard two-compartment model, except that the fitted inner compartment volume was found to be the true distribution volume. In any case, extreme caution is indicated in applying multicompartment models to systems where the binding characteristics are unknown, since nonlinear binding of drug to protein may mimic multicompartment distribution quite closely.

Although, in the case of drugs that bind to serum albumin, binding sites may be present in excess, in several systems a drug is bound to a specific carrier protein present in low concentration in the plasma. Concentration–time curves for two such compounds—aldosterone and thyroxine—were calculated, using literature values for the binding parameters and assuming doses within a reasonable pharmacologic range. To simplify calculations, it was assumed that aldosterone is bound only to transcortin and that thyroxine is bound only to thyroxine-binding globulin, ignoring binding to other plasma proteins. A half-life of 30 min. is arbitrarily assumed for free drug in both cases. These calculations are an attempt to model only one facet of the pharmacokinetics of these substances. Such important features as true elimination rates and rates of endogenous synthesis are not considered here; hence, the calculated curves are best used

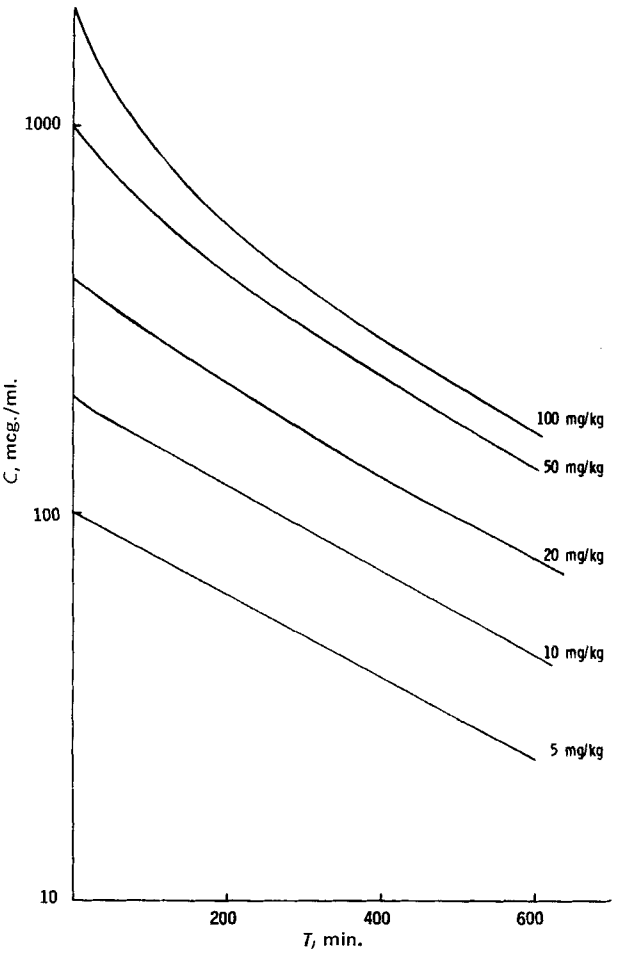


Figure 2—*Calculated concentration–time curves in plasma of the drug illustrated in Fig. 1, assuming 10 binding sites per albumin molecule. Doses are shown with each curve, and other assumptions are given in the text.*

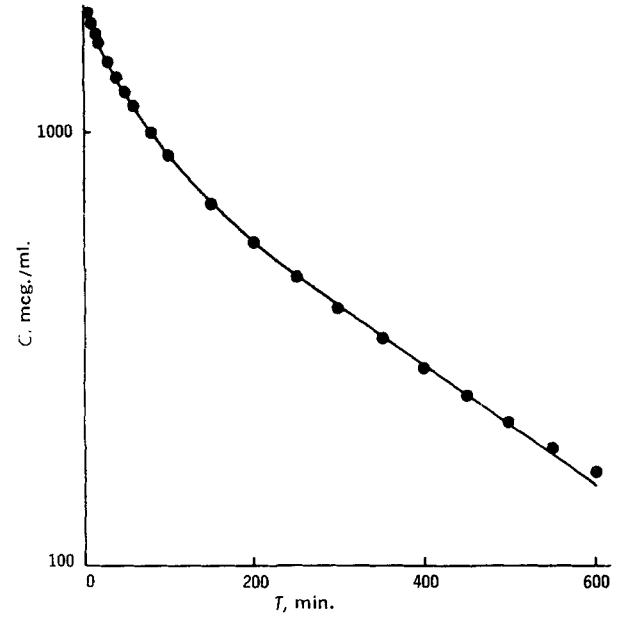


Figure 3—*Fitting of the 100-mg./kg. curve of Fig. 2 to a two-compartment model without binding. Points are taken from Fig. 2; the smooth curve is the least-squares fit to the two-compartment model. Fitted values of the apparent constants are: distribution half-time = 120 min., elimination half-time = 130 min., inner compartment volume = 50 ml./kg., and outer compartment volume = 25 ml./kg.*

merely to point out some possible features of the systems to which plasma protein binding may contribute.

For aldosterone, the assumed values (9) are:

1. Transcortin concentration = 2.5 mg./100 ml.
2. Transcortin molecular weight = 45,000.
3. Association constant for single binding site = 6.5×10^6.
4. Aldosterone molecular weight = 360.

Calculated concentration–time curves for aldosterone doses of 0.02–0.2 mg. are shown in Fig. 4. While a slight curvature is discernible at the high doses, experimental data would be expected to fall almost on a straight line. For all practical purposes, despite the limited amount of transcortin present in plasma, aldosterone can be expected to fall within the range where the binding relationship is linear at reasonable dose levels.

Thyroxine, on the other hand, appears to exceed the binding capacity of thyroxine-binding globulin at high doses. The assumed values (8) are:

1. Thyroxine-binding globulin concentration = 2 mg./100 ml.
2. Thyroxine-binding globulin molecular weight = 50,000.
3. Association constant for single binding site = 5×10^9 (*i.e.*, thyroxine 99% bound in the range where the binding relationship is a linear one).
4. Thyroxine molecular weight = 777.

Curves for doses of 0.2 and 2 mg. are shown in Fig. 5. The higher dose shows extremely sharp curvature in the concentration–time curve; in fact, unless measurements are made at very early times, it would be quite easy to miss the nonlinear portion of the curve. If only the linear portion is seen, extrapolated zero-time concentrations would be far lower than the true initial concentration of thyroxine, and the distribution volume would appear to increase with increasing dose. It must be reemphasized that, because of the arbitrary choice of an elimination half-time for aldosterone and thyroxine, the curves in Figs. 4 and 5 could not be expected to fit the true situations exactly. However, the general shape of these curves should indicate some points of interest and significance in the expected pharmacokinetics of these substances.

RESULTS AND CONCLUSIONS FOR A TWO-COMPARTMENT MODEL WITH BINDING

The effect of plasma protein binding on the distribution of drugs into tissues has been the subject of much consideration. In qualitative terms, it was often noted that an increase in the proportion of free drug in plasma results in more rapid elimination and in a rise

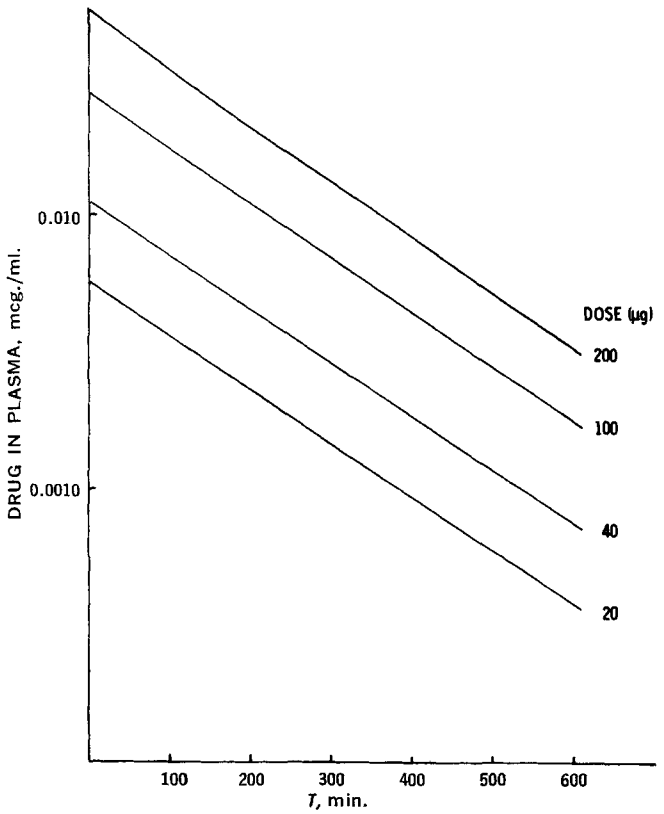


Figure 4—*Calculated concentration–time curves in plasma of aldosterone, using the assumed values given in the text. Doses are shown with each curve.*

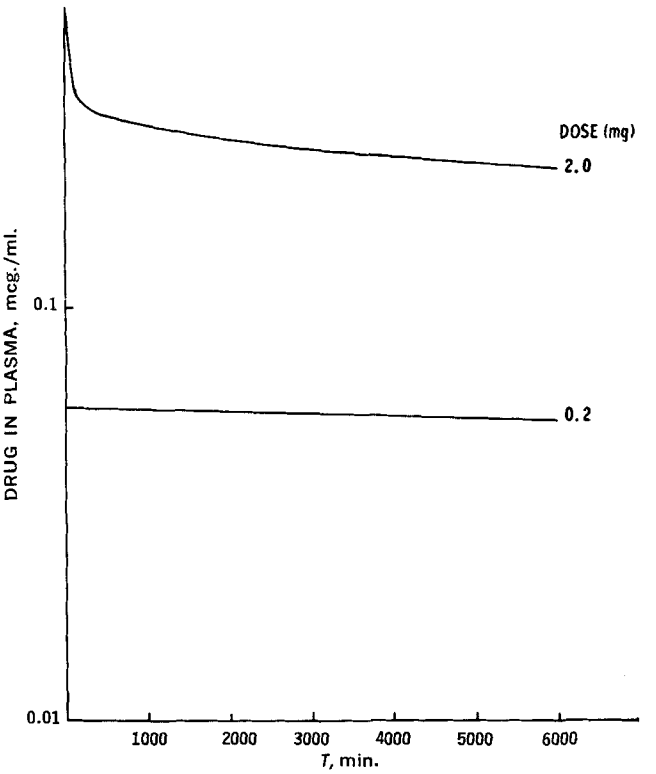


Figure 5—*Calculated concentration–time curves in plasma of thyroxine, using the assumed values given in the text. Doses are shown with each curve.*

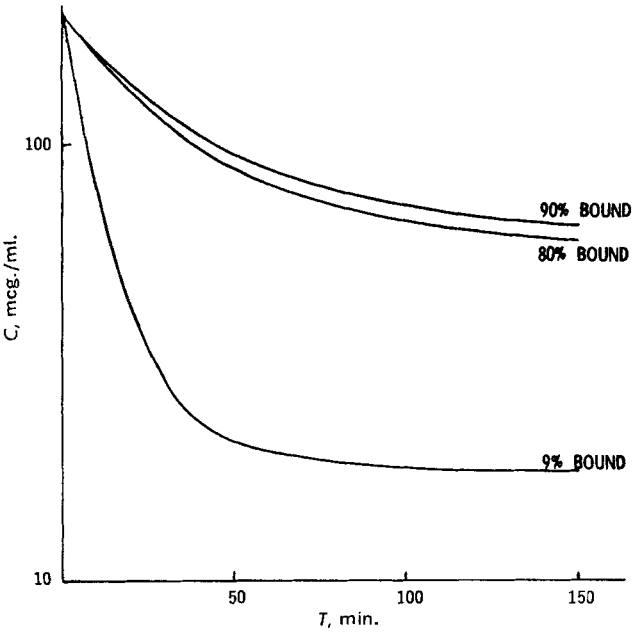


Figure 6—*Calculated concentration–time curves in plasma of a 10-mg./kg. dose of hypothetical drug of mol. wt. 150 in the two-compartment system. The inner distribution volume is 50 ml./kg.; the outer is 600 ml./kg. The proportion of binding in the linear phase is shown with each curve.*

in drug concentration in tissues (10, 17, 23). A decrease in the proportion of bound drug from 90 to 80%, for example, doubles the plasma concentration of free drug. Thus, it was suggested (10) that even fairly small competitive effects could be extremely important in changing drug levels in tissues.

This mechanism of changing tissue levels of drug, however, may actually be limited to a relatively small number of special cases. The crucial variable is the ratio of tissue distribution volume to plasma volume—in the notation used for the two-compartment case, V_2/V_1. If V_2/V_1 is large, the total amount of drug liberated from plasma proteins, even with relatively large shifts in binding, is insufficient to cause a large concentration change in the tissue volume into which it is diluted.

This point is illustrated using the hypothetical drug of mol. wt. 150 whose behavior in the one-compartment system was already described. In addition to the previous assumptions, a distribution half-time of 5 min. and an outer compartment volume of 600 ml./kg., a reasonable estimate for nonplasma body water, were assumed. The dose is 10 mg./kg., and there are 10 binding sites per serum albumin molecule. The computer program used to calculate concentration–time curves from the two-compartment model does not incorporate the equations for binding competition. Therefore, three sets of independent calculations were carried out, each assuming a different binding constant, to show the effect of changing a system in which drug is 90% bound in the region where the linear binding relationship of Eq. 3 holds to one in which drug is 80 or 9% bound.

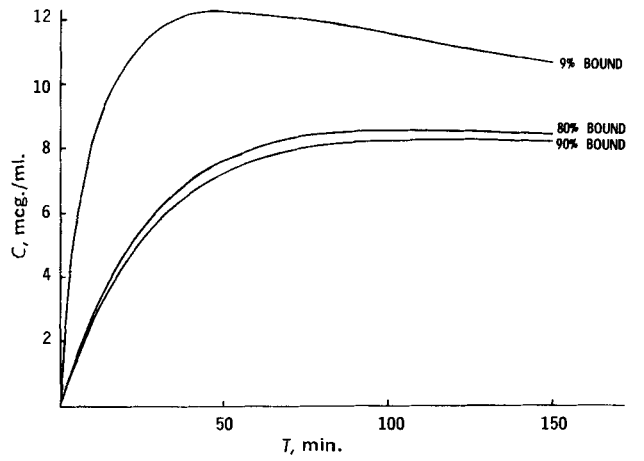


Figure 7—*Calculated concentration–time curves in the tissue compartment for a 10-mg./kg. dose of a hypothetical drug of mol. wt. 150, corresponding to the plasma curve of Fig. 6.*

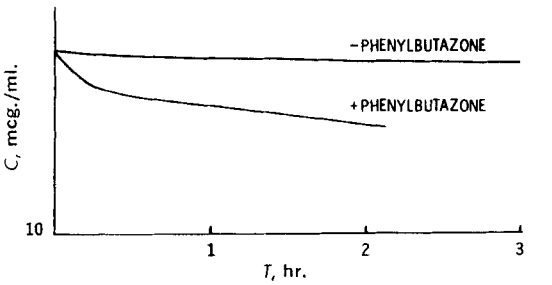


Figure 8—*Calculated concentration–time curves in plasma of warfarin in the presence and absence of phenylbutazone, using the assumptions presented in the text.*

The concentration–time curves for plasma are shown in Fig. 6. Considerable curvature is noticeable at a 10-mg./kg. dose of this hypothetical drug because of the phase corresponding to distribution into tissue. A doubling of free drug concentration, as the proportion of binding is dropped from 90 to 80%, produces very little change in the plasma kinetics, while a more dramatic shift to 9% binding greatly increases the rate of disappearance from plasma. The situation calculated for the tissue compartment is shown in Fig. 7. Because of the large value of V_2/V_1, even the shift from 90 to 9% binding produces less than a doubling of tissue concentration (except at very early times), while the increase due to the shift from 90 to 80% bound is very slight. Thus, if a drug is redistributed into body water, it seems unlikely that binding competition, no matter how effective, could cause large increases in tissue concentration.

Nonetheless, there are several examples in the literature where displacement of bound drug is thought to have major effects. One of the best known is the case of warfarin and phenylbutazone (3, 4). Administration of phenylbutazone decreases the half-life of warfarin but potentiates its anticoagulant activity. O'Reilly and Levy (4) showed that the effect of phenylbutazone is on the relationship between the synthesis of prothrombin complex activity and total concentration of warfarin in plasma. They concluded that phenylbutazone increases the proportion of free warfarin in plasma, making it more available to its pharmacologic receptor site.

The quantitative basis of this effect may be explored using the data of Aggeler *et al.* (3). Extrapolation of the warfarin concentration–time curves in plasma yields an estimated total distribution volume of only 100 ml./kg. If one assumes that warfarin can be fitted to a two-compartment model with inner and outer compartment volumes both equal to 50 ml./kg., it becomes apparent that the dilution factor due to V_2/V_1 is quite small.

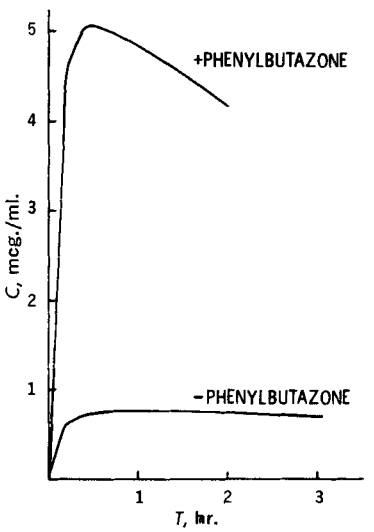


Figure 9—*Calculated concentration – time curves in the tissue compartment of warfarin in the presence and absence of phenylbutazone, corresponding to the plasma curves of Fig. 8.*

Calculations of predicted warfarin concentration–time curves were carried out using the following values (3):

1. Association constant for single binding site = 6.65×10^4.
2. Association constant in the presence of phenylbutazone = 7×10^3.
3. Distribution half-time = 5 min.
4. Inner and outer compartment volumes = 50 ml./kg.
5. Elimination half-time of total drug in the region where the binding relationship is linear = 44.8 hr.
6. Initial dose = 1.5 mg./kg.
7. Warfarin molecular weight = 308.

Because of the strong tendency of the successive approximation procedure to generate concentrations that become nonmonotonic with time, the calculated concentration–time curves (Figs. 8 and 9) are of only very short duration, barely sufficient, in fact, to illustrate the elimination of drug from either plasma or tissues. Even so, the effect of phenylbutazone on the pharmacokinetics of warfarin is quite apparent. The half-life of warfarin in plasma (Fig. 8) is somewhat decreased in the presence of phenylbutazone, but the most dramatic effect is on the warfarin concentration in the outer compartment (Fig. 9). It seems clear that the low value of V_2/V_1 is sufficient to give rise to a large increase in tissue concentration of warfarin in the presence of phenylbutazone.

Other special cases in which binding competition is of great importance may arise from other circumstances. The sulfonamide-related kernicterus of the newborn (12–15) appears to be due to binding competition between sulfonamides and endogenous bilirubin. The data on this system seem not to be amenable to simple pharmacokinetic analysis at the present time. Josephson and Furst (15) report, for example, that bilirubin appears to be more tightly bound to serum albumin than sulfamethoxypyridazine or sulfamoxole, yet the sulfa drugs appear to displace bilirubin in competitive binding experiments while bilirubin does not displace the sulfa drugs. Unless the apparent contradictions in these data are resolved by direct determination of binding constants, it will not be possible to model the bilirubin–sulfonamide system. Even if V_2/V_1 is quite large for bilirubin, the continuous endogenous synthesis of the compound could still give rise to a large increase in tissue bilirubin concentration by titrating the patient, so to speak, up to a new steady-state level. This titration effect, which would not be possible with a single dose of an exogenous drug, may be responsible for the severity of the sulfonamide–bilirubin interaction.

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