

Simple Model to Explain Effects of Plasma Protein Binding and Tissue Binding on Calculated Volumes of Distribution, Apparent Elimination Rate Constants and Clearances*

J. G. Wagner

College of Pharmacy and Upjohn Center for Clinical Pharmacology,
The University of Michigan, Ann Arbor, Michigan, USA

Received: March 26, 1976, accepted: June 10, 1976

Summary. A simple pharmacokinetic model, incorporating linear plasma protein binding, linear tissue binding, and first order elimination of free (unbound) drug, was studied. If Cl_p is the plasma clearance, V_f is the "true" volume of distribution of free drug, β is the apparent elimination rate constant, σ is the fraction of the drug which is free in plasma, f is the fraction of the drug which is free in the entire body, k_f is the intrinsic elimination rate constant for free drug, and A_{TB} is the initial amount of drug which is bound to tissues, then the model indicates that the following relationships hold: (1) $Cl_p = V_f \sigma k_f$; (2) $\beta = f k_f$; and $V_{dext} = (\sigma/f) V_f$. Only σ , and not f , can be measured *experimentally*. Dividing Cl_p by σ provides an estimate of the intrinsic clearance of free drug, $V_f k_f$. A plot of V_{dext} versus σ has an intercept equal to V_f , and the ratio of the slope/intercept is an estimate of A_{TB}/A_f , where A_f is the initial amount of free drug (equal to V_f times initial concentration of free drug in plasma). Thus, an estimate of A_{TB} may be obtained. Dividing the intrinsic clearance by V_f provides an estimate of k_f . Thus, theoretically, estimates of V_f , k_f , A_{TB} and f may be obtained. The variables are not separated when β is plotted versus σ , and curvature of such plots is expected; no useful information is obtained from such plots.

Key words: Plasma protein binding, tissue binding, volume of distribution of free drug, intrinsic rate constant for free drug, amount bound to tissues.

There has been much discussion in the literature on the effects of plasma protein binding on the distribution, elimination, and activity of drugs (Anton and Solomon, 1973; Benya and Wagner, 1975; Coffey, 1972; Garrett, 1972; Keen, 1972; Krüger-Thiemer, 1966 and 1968; Levy and Yacobi, 1974; Martin, 1965; Wagner, 1971 and 1975) and these citations are not intended to be complete. The review of Keen (1971) not only discussed the

possible effects of plasma protein binding, but also discussed the possible effects of tissue binding. He cited data showing that cardiac glycosides which are highly bound to plasma proteins are also highly bound to tissues. A similar situation has been reported for diphenhydramine (Wagner, 1973 and Albert et al., 1975) and for warfarin in the rat (Benya and Wagner, 1975; Yacobi and Levy, 1975).

Several authors (Coffey, 1972; Garrett, 1972; Keen, 1971; Krüger-Thiemer, 1966 and 1968; Levy and Yacobi, 1974; Martin, 1965; Yacobi and Levy, 1975) have developed mathematical and pharmacokinetic models and equations which incorporate plasma protein binding. Others (DiSanto, 1971 and Wagner, 1971 and 1975) have developed models to illustrate possible ef-

*Partly supported by Public Health Service Grant 5-P-11-GM 1559 and partly by Grant 1R01AA00683-01A1 from the National Institute on Alcohol Abuse and Alcoholism, Rockville, Maryland.

fects of tissue binding. Bischoff and Dedrick (1968) developed a flow rate-limited model, which incorporated both plasma protein binding and binding to several different types of tissues; they assumed that the relationship for binding to plasma proteins could also be used for tissue binding; equations used were those of the Langmuir type. Hermann (1975) showed that the binding of ouabain and digitoxin by human erythrocytes obeyed the Langmuir equation. When the dissociation constant is much greater than the drug concentration, the Langmuir equation collapses to a linear binding equation.

In inter-individual comparisons of a group of one species of animal or man it would be advantageous if one could predict, for example, the plasma clearance of a drug by measurement of the fraction of the drug which is free in plasma. The question arises as to which are the appropriate pharmacokinetic parameters to correlate with this fraction. The purpose of this report is to determine which relationship or relationships have a firm footing in pharmacokinetic theory, rather than the alternative approach of just making empirical correlations. The physical model approach also suggests when one might expect a trend line to be linear or curved; often, scatter in data, as a result of just biological variation and assay error, does not allow one to adequately determine when a trend line should be linear or curved.

THEORETICAL

Symbolism

- A_f - amount (moles) of free drug at time t .
 A_f^0 - initial amount (moles) of free drug at time zero.
 A_{PB} - amount (moles) of plasma protein bound drug at time t .
 A_{PB}^0 - initial amount (moles) of plasma protein bound drug at time zero.
 A_{TB} - amount (moles) of tissue bound drug at time t .
 A_{TB}^0 - initial amount (moles) of tissue bound drug at time zero.
 β - apparent elimination rate constant, such that $-d \ln C_p/dt = \beta$
 Cl_p - plasma clearance (defined by equation 31).
 C_f - molar concentration of free (unbound) drug at time t .
 C_f^0 - initial molar concentration of free (unbound) drug at time zero.
 C_p - molar total plasma concentration of drug (defined by equation 24) at time t .
 C_p^0 - initial molar total plasma concentration of drug at time zero.
 C_{PB} - molar concentration of plasma protein bound drug at time t .

- C_{TB} - molar concentration of tissue bound drug at time t .
 D - the dose of drug administered by bolus intravenous injection.
 f - the fraction of the total amount of drug in the body which is free. (defined by equation 14).
 k_f - the intrinsic elimination rate constant for free drug (dimension of 1/time).
 K_p - the association constant for protein bound drug ($K_p = k_1/k_2$).
 K_T - the association constant for tissue bound drug ($K_T = k_3/k_4$).
 m - the number of binding sites for drug on tissue protein.
 n - the number of binding sites on plasma protein.
 P - molar concentration of plasma protein.
 σ - the fraction of drug which is free in plasma (defined by equation 19).
 t - time.
 t' - some specific time.
 t_1 - a lag time needed in real life to establish model conditions.
 T - molar concentration of tissue which binds the drug.
 V_{darea} - a volume of distribution defined by equation 34.
 V_{dext} - a volume of distribution defined by equation 39.
 V_f - the "true" volume of distribution of free drug (dimension of liters).
 V_T - the volume of tissue which binds drug (liters).

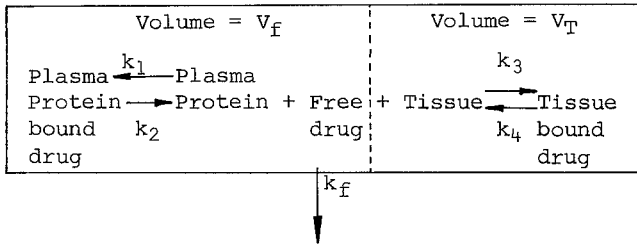
Assumptions

1. When the dose, D , is given by bolus intravenous injection, the drug instantaneously is partitioned into three portions, namely A_{TB}^0 , A_{PB}^0 , and A_f^0 .
2. Binding to both plasma protein and tissue is linear and independent of drug concentration.
3. The volume occupied by plasma proteins is ignored and hence $A_{PB} = V_f C_{PB}$ and $A_f = V_f C_f$.
4. The free drug is eliminated according to first order kinetics with rate constant equal to k_f .
5. Only free drug is available for elimination.

Model

The simple model includes only binding of drug to one class of sites on one plasma protein, binding of drug to one class of sites on one type of tissue and one fluid volume. However, the approach taken with the simple model could be readily extended to more complicated models as was done by DiSanto (1971) and Wagner (1971) with analogous nonlinear models. The conclusions reached with the simple model would be extrapolatable to

more complicated models of a similar type. The model is shown schematically in Scheme 1.



Scheme 1. Modified "one compartment open model" incorporating linear tissue binding and linear plasma protein binding

Equations

For linear binding:

$$C_{PB} = n K_P P C_f \quad \text{Eq. (1)}$$

$$A_{PB} = V_f C_{PB} = n K_P P V_f C_f \\ = n K_P P A_f \quad \text{Eq. (2)}$$

$$C_{TB} = m K_T T C_f \quad \text{Eq. (3)}$$

$$A_{TB} = V_T C_{TB} = m K_T T V_T C_f \\ = m K_T T \frac{V_T}{V_f} A_f \quad \text{Eq. (4)}$$

$$A_f = V_f C_f \quad \text{Eq. (5)}$$

Mass balance gives:

$$A_f + A_{PB} + A_{TB} + k_f \int_0^{t'} A_f dt = D \\ \text{Eq. (6)}$$

In equation 6, the term containing the integral represents the amount of drug eliminated between time zero and time t' .

Differentiation of equation 6 with respect to time yields equation 7.

$$\frac{dA_f}{dt} + \frac{dA_{PB}}{dt} + \frac{dA_{TB}}{dt} + k_f A_f = 0 \quad \text{Eq. (7)}$$

Now,

$$\frac{dA_{PB}}{dt} = \frac{dA_{PB}}{dA_f} \cdot \frac{dA_f}{dt} = (n K_P P) \frac{dA_f}{dt} \\ \text{Eq. (8)}$$

$$\frac{dA_{TB}}{dt} = \frac{dA_{TB}}{dA_f} \cdot \frac{dA_f}{dt} = (m K_T T \frac{V_T}{V_f}) \frac{dA_f}{dt} \\ \text{Eq. (9)}$$

Substituting from equations 8 and 9 into equation 7 yields:

$$\frac{dA_f}{dt} [1 + n K_P P + m K_T T \frac{V_T}{V_f}] + k_f A_f = 0 \\ \text{Eq. (10)}$$

Rearrangement of equation 10 gives equation 11.

$$-\frac{dA_f}{dt} = \left[\frac{k_f}{1 + n K_P P + m K_T T \frac{V_T}{V_f}} \right] A_f \\ \text{Eq. (11)}$$

Substituting from equation 5 for A_f and cancelling the V_f 's on both sides yields equation 12 from equation 11.

$$-\frac{dC_f}{dt} = \left[\frac{k_f}{1 + n K_P P + m K_T T \frac{V_T}{V_f}} \right] C_f \\ = \beta C_f \quad \text{Eq. (12)}$$

where β is the apparent elimination rate constant and is given by equation 13.

$$\beta = \frac{k_f}{1 + n K_P P + m K_T T \frac{V_T}{V_f}} \\ \text{Eq. (13)}$$

If we define f as the fraction of the total drug in the body which is free, then:

$$f = \frac{A_f}{A_f + A_{PB} + A_{TB}} = \frac{1}{1 + \frac{A_{PB}}{A_f} + \frac{A_{TB}}{A_f}} \\ \text{Eq. (14)}$$

From equation 2 we obtain:

$$\frac{A_{PB}}{A_f} = n K_P P \quad \text{Eq. (15)}$$

From equation 4 we obtain:

$$\frac{A_{TB}}{A_f} = m K_T T \frac{V_T}{V_f} \quad \text{Eq. (16)}$$

Substituting from equations 15 and 16 into equation 14 gives equation 17.

$$f = \frac{1}{1 + n K_P P + m K_T T \frac{V_T}{V_f}} \quad \text{Eq. (17)}$$

Substituting from equation 17 into equation 13 gives equation 18.

$$\beta = f k_f \quad \text{Eq. (18)}$$

Thus, according to this simple model, the apparent elimination rate constant, β , is a product of the intrinsic elimination rate constant for free drug, k_f , and the fraction of the total amount of drug in the body which is free, f .

If we define σ as the fraction of the drug which is free (unbound) in the volume V_f (and plasma is assumed to be representative of this fluid), then:

$$\begin{aligned}\sigma &= \frac{C_f}{C_f + C_{PB}} = \frac{V_f C_f}{V_f C_f + V_f C_{PB}} \\ &= \frac{A_f}{A_f + A_{PB}} = \frac{1}{1 + \frac{A_{PB}}{A_f}}\end{aligned}\quad \text{Eq. (19)}$$

Thus,

$$1 + \frac{A_{PB}}{A_f} = \frac{1}{\sigma} \quad \text{Eq. (20)}$$

Substituting from equation 20 into equation 14 and rearranging gives:

$$f = \frac{\sigma}{1 + \left(\frac{A_{TB}}{A_f}\right)\sigma} \quad \text{Eq. (21)}$$

Substituting for f in equation 21 gives equation 22.

$$\beta = \left[\frac{\sigma}{1 + \left(\frac{A_{TB}}{A_f}\right)\sigma} \right] k_f \quad \text{Eq. (22)}$$

Equation 22 indicates that for inter-individual comparisons of a drug in one species of animal or in man, a plot of the apparent elimination rate constant, β , versus the fraction of drug which is free in plasma, σ , will be gently curved over a small range of σ values, even when the ratio A_{TB}/A_f is constant from individual to individual of the species. With the scatter expected in such a plot (because of expected non-constancy of the A_{TB}/A_f ratios) such curvature would be difficult to detect with real data.

Integration of equation 12 yields equation 23.

$$C_f = C_f^0 e^{-\beta t} \quad \text{Eq. (23)}$$

The total plasma concentration, C_p , is given by equation 24, after substituting from equation 1 for C_{PB} .

$$C_p = C_f + C_{PB} = (1 + n K_p P) C_f \quad \text{Eq. (24)}$$

Multiplying both sides of equation 23 by $(1 + n K_p P)$ and utilizing equation 24 gives equation 25.

$$C_p = (1 + n K_p P) C_f^0 e^{-\beta t} = C_p^0 e^{-\beta t} \quad \text{Eq. (25)}$$

where

$$C_p^0 = (1 + n K_p P) C_f^0 \quad \text{Eq. (26)}$$

Hence, the model predicts that the apparent elimination rate constant, β , obtained from total plasma concentrations is the same as that obtained from free drug concentrations.

The initial condition at time zero for the model is given by equation 27.

$$A_f^0 + A_{TB}^0 + A_{PB}^0 = D \quad \text{Eq. (27)}$$

In equation 27, A_f^0 , A_{TB}^0 , and A_{PB}^0 are the initial amounts existing as free drug, drug bound to tissues, and drug bound to plasma proteins, respectively, and D is the dose administered. In real life it would take some finite time, even when the values of k_1 and k_3 are large, to attain this "initial condition", but this lag time, t_1 , would most probably be small relative to the measured time courses of most drugs. The model assumes such partitioning, as indicated by equation 27, has already occurred at time zero. Hence, in real life the only difference would be that the D in equation 27 would be replaced by A_b^0 , the apparent initial amount of drug in the body, such that $A_b^0 < D$, and t would be replaced by $(t - t_1)$.

An estimate would be

$$A_b^0 = D - V_f k_f \int_0^{t_1} C_f dt.$$

It may be readily seen that C_p^0 is also given by equation 28.

$$C_p^0 = \left[\frac{A_f^0 + A_{PB}^0}{A_f^0 + A_{PB}^0 + A_{TB}^0} \right] \frac{D}{V_f} \quad \text{Eq. (28)}$$

Equations 14 and 19 also apply to the initial condition, as well as at any time t , hence substituting from those equations and cancelling the A_f^0 's yields equation 29.

$$\frac{f}{\sigma} = \frac{A_f^0 + A_{PB}^0}{A_f^0 + A_{PB}^0 + A_{TB}^0} \quad \text{Eq. (29)}$$

Substituting from equation 29 into equation 28 gives equation 30.

$$C_p^0 = \left(\frac{f}{\sigma}\right) \left(\frac{D}{V_f}\right) \quad \text{Eq. (30)}$$

The plasma clearance, Cl_p , is usually calculated as the ratio of the dose to the total area under the total plasma concentration-time curve, as indicated by equation 31.

$$Cl_p = \frac{D}{\int_0^{\infty} C_p dt} \quad \text{Eq. (31)}$$

Integration of equation 25 between the limits of $t = 0$ and $t = \infty$, followed by substitution for C_p^0 from equation 30, substitution for β from equation 18 and cancellation of the f 's gives equation 32.

$$\int_0^{\infty} C_p dt = \frac{C_p^0}{\beta} = \frac{D}{V_f \sigma k_f} \quad \text{Eq. (32)}$$

Substituting for the integral in equation 31 from equation 32 and cancellation of the D's yields equation 33.

$$Cl_p = V_f \sigma k_f \quad \text{Eq. (33)}$$

Thus, according to the model, the calculated plasma clearance, Cl_p , is the product of the intrinsic clearance of free drug, $V_f k_f$, and the fraction of the drug which is free in plasma, σ . For inter-individual comparisons a plot of Cl_p versus σ should yield a straight line, passing through the origin, with slope equal to $V_f k_f$, providing that the intrinsic clearance of free drug is constant from one individual to the next. Alternatively, and preferred, the plasma clearance of each individual may be divided by the fraction of drug free in plasma for that individual to obtain individual estimates of the intrinsic clearance, then these analyzed statistically to obtain a measure of dispersion.

V_{darea} is usually calculated with equation 34.

$$V_{darea} = \frac{D}{\beta \int_0^\infty C_p dt} = \frac{Cl_p}{\beta} \quad \text{Eq. (34)}$$

Substituting from equation 18 for β and from equation 33 for Cl_p into equation 34, and cancellation of the k_f 's, gives equation 35.

$$V_{darea} = \left(\frac{\sigma}{f}\right) V_f \quad \text{Eq. (35)}$$

Equation 35 indicates that in the absence of both plasma protein binding and tissue binding (i.e. $\sigma = f = 1$), $V_{darea} = V_f$. However, equation 35 also indicates that V_{darea} is not a simple function of σ , as is clarified below.

Rearrangement of equation 27 gives equation 36.

$$A_f^0 = D - A_{TB}^0 - A_{PB}^0 \quad \text{Eq. (36)}$$

Substituting from equation 27 and 36 into equation 14, and rearrangement yields equation 37.

$$f = 1 - \frac{A_{TB}^0}{D} - \frac{A_{PB}^0}{D} \quad \text{Eq. (37)}$$

Substituting for f from equation 37 into equation 35 yields equation 38.

$$V_{darea} = \left[\frac{\sigma}{1 - \frac{A_{TB}^0}{D} - \frac{A_{PB}^0}{D}} \right] V_f \quad \text{Eq. (38)}$$

The portion of equation 38 marked off by square brackets can have a value ranging from much less than unity to much greater than unity, depending upon the relative degrees of plasma protein bind-

ing and tissue binding of the drug. When plasma protein binding is very low (i.e. $\sigma \rightarrow 1$ and $A_{PB}^0/D \rightarrow 0$), and tissue binding is large (i.e. $A_{TB}^0/D \rightarrow 1$), then this bracketed portion will be much larger than unity, and $V_{darea} \gg V_f$. If $\sigma = 0.5$ and $(A_{TB}^0 + A_{PB}^0)/D = 0.5$, then the bracketed portion would be equal to unity and $V_{darea} = V_f$. Even for a drug which is both very highly plasma protein bound and tissue bound, V_{darea} could be equal to V_f ; for example, if $\sigma = 0.02$ and $(A_{TB}^0 + A_{PB}^0)/D = 0.98$, then the bracketed portion would be equal to unity. Usually, of course, the bracketed portion would not be equal to unity, hence either $V_{darea} > V_f$ or $V_{darea} < V_f$. Thus, the calculated volume of distribution is not equal, usually, to the volume of distribution of free drug.

The extrapolated volume of distribution, V_{dext} , is calculated with equation 39.

$$V_{dext} = \frac{D}{C_p^0} \quad \text{Eq. (39)}$$

Substituting for C_p^0 from equation 30 into equation 39 yields equation 40.

$$V_{dext} = \left(\frac{\sigma}{f}\right) V_f \quad \text{Eq. (40)}$$

By comparing the right hand sides of equations 35 and 40 we see that for this model:

$$V_{dext} = V_{darea} \quad \text{Eq. (41)}$$

Equation 41 is the expected result for this modified "one compartment open model". If there was more than one fluid compartment and/or distribution to some tissues took an appreciable time, than a multicompartmental model would be required, and, in such cases, $V_{dext} > V_{darea}$.

From equations 14 and 19 it may readily be shown that:

$$\frac{\sigma}{f} = 1 + \left(\frac{V_T}{V_f}\right) \left(\frac{C_{TB}}{C_p}\right) \quad \text{Eq. (42)}$$

Equation 42 indicates that $\sigma \geq f$. Also, rearrangement of equation 34 gives equation 43. Hence, the model agrees with classical concepts.

$$Cl_p = V_{darea} \beta \quad \text{Eq. (43)}$$

Keen (1971) recommended that the volume of distribution be calculated from free drug concentrations, rather than from total drug concentrations. If this is done, then the volume calculated, based on the simple model, is V_f/f . The same result would be obtained by dividing the volumes, V_{dext} and V_{darea} , estimated from total drug concentrations, by σ . It should be noted that such volumes

are still dependent upon relative degrees of plasma protein binding and tissue binding, as pointed out by Keen (1971). The product of the apparent elimination rate constant, β , and such a volume (estimated from free drug concentrations) is, by use of equation 18, equal to the intrinsic clearance of free drug, as indicated by equation 44.

$$\begin{aligned} \text{Intrinsic clearance} &= \left(\frac{V_f}{f}\right) (f k_f) \\ &= V_f k_f \end{aligned} \quad \text{Eq. (44)}$$

If there are multicompartmental pharmacokinetics, then the volume calculated from free drug concentrations should be the modified $V_{d\text{area}}$, rather than $V_{d\text{ext}}$, unless, as is sometimes the case, the two volumes are essentially identical.

Estimation of V_f and A_{TB}/A_f - Substituting from equation 20 into equation 14 yields equation 45. The latter is also obtained by rearranging equation 21.

$$f = \frac{1}{\frac{1}{\sigma} + \frac{A_{TB}}{A_f}} \quad \text{Eq. (45)}$$

Substituting from equation 45 into equation 40 and simplifying gives equation 46.

$$V_{d\text{ext}} = \left[1 + \sigma \left(\frac{A_{TB}}{A_f}\right)\right] V_f \quad \text{Eq. (46)}$$

Equation 46 indicates that a plot of $V_{d\text{ext}}$ versus σ will be linear with intercept equal to V_f , and slope equal to $(A_{TB}/A_f) V_f$. Hence, A_{TB}/A_f is obtained with equation 47.

$$\frac{A_{TB}}{A_f} = \frac{\text{Slope}}{\text{Intercept}} = \frac{(A_{TB}/A_f) V_f}{V_f} \quad \text{Eq. (47)}$$

Estimation of k_f and f - Since V_f is known (see above) and the intrinsic clearance, $V_f k_f$, is known by dividing Cl_p by σ (equation 33), then an estimate of k_f is obtained from equation 48.

$$k_f = \frac{\text{Intrinsic Clearance}}{V_f} = \frac{V_f k_f}{V_f} \quad \text{Eq. (48)}$$

Now that k_f and β are known, then "f" may be estimated by rearranging equation 18 to give equation 49.

$$f = \frac{\beta}{k_f} \quad \text{Eq. (49)}$$

Estimation of A_{TB}^0 - If we write equation 5 for the zero time condition, then equation 50 is obtained. Also, since binding is linear, equation 51 holds.

$$A_f^0 = V_f C_f^0 \quad \text{Eq. (50)}$$

$$\frac{A_{TB}}{A_f} = \frac{A_{TB}^0}{A_f^0} \quad \text{Eq. (51)}$$

Since the ratio A_{TB}^0/A_f^0 is known from equations 47 and 51, and A_f^0 is known from equation 50, then A_{TB}^0 is obtained with equation 52.

$$A_{TB}^0 = \frac{A_{TB}/A_f}{V_f C_f^0} \quad \text{Eq. (52)}$$

SIMULATIONS

Simulation with equation 22 - A plot of the apparent elimination rate constant, β , versus the fraction free in plasma, σ , does not separate the variables as may be seen from equation 22. Even in those cases where the ratio A_{TB}/A_f is constant, while σ varies, plots of β versus σ will be curved as may be seen in Figure 1. The greater the value of the ratio A_{TB}/A_f , the greater the curvature. For low values of A_{TB}/A_f , the plots are nearly linear. In real life, one would expect A_{TB}/A_f to vary, and not be constant, from one individual to the next. This would cause scatter on the plots of β versus σ . Evaluation of such "scattered data" by linear regression analysis, would lead to an apparent positive intercept on the ordinate. But, the intercept and slope values obtained from such plots would have no physical significance. Thus, correlations of β with σ are not recommended. This would also apply to any transformation, such as half-life.

Simulation with equation 46 - Two sets of data were generated by letting A_{TB}/A_f equal 10 and 50, then varying σ over the range 0.004 to 0.02. For a given value of σ , the value of "f" was calculated with equation 45, then the corresponding value of σ/f , equivalent to $V_{d\text{ext}}/V_f$, was calculated using equation 40. Results are shown in Figure 2. Extrapolation of the linear plots to the ordinate yielded a value of unity, equal to V_f/V_f in this case. The slopes of the lines are the same as the assigned values of A_{TB}/A_f , namely 50 and 10. Suppose the value of V_f was 0.6 l/kg. Then, using the value of $A_{TB}/A_f = 50$, the slope of the $V_{d\text{ext}}$ versus σ plot would be 30, and the intercept would be 0.6. Hence, the calculated value of A_{TB}/A_f would be slope/intercept = 30/0.6 = 50. If there was considerable scatter on a plot of $V_{d\text{ext}}$ versus σ , this would indicate variation in the ratio A_{TB}/A_f from one individual to the next.

DISCUSSION

The simple model employed incorporated linear tissue binding, linear plasma protein binding, and first order elimination

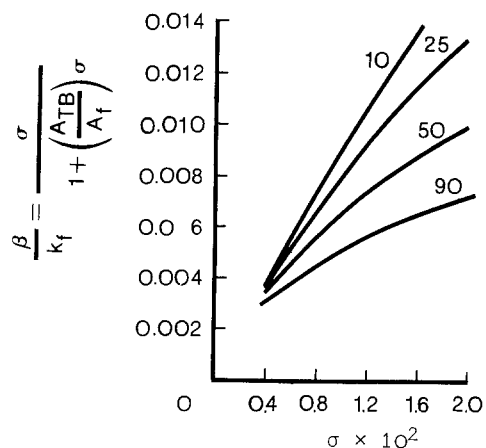


Fig. 1. Results of simulations with equation 22 (see text). Indicates curvature obtained when apparent elimination rate constant plotted versus fraction free in plasma. Numbers on curves refer to assigned values of the ratio A_{TB}/A_f

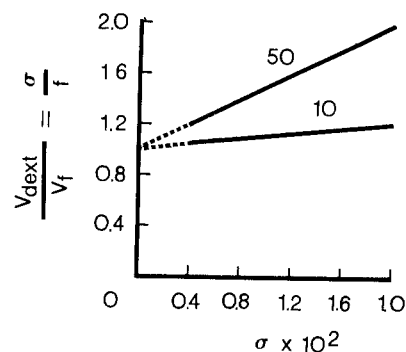


Fig. 2. Results of simulations with equation 40 (see text). Numbers on lines refer to assigned values of the ratio A_{TB}/A_f

of free (unbound) drug, hence any conclusions are limited to real systems with these properties. The most important equations derived are 18, 22, 33, 40, and 46 through 52, which require equations 14, 19, 31, 34 and 39 for interpretation.

With respect to inter-individual comparisons, the model suggests the following:

(1) Dividing the plasma clearance, Cl_p , (estimated from total plasma concentrations) by the fraction of drug free in plasma, σ , will provide estimate of the intrinsic clearance of free drug, $V_f k_f$. The values obtained from a panel of subjects or patients, or a group of animals, may then be treated statistically to obtain a mean and some measure of dispersion, such as the coefficient of variation. Alternatively, one could plot Cl_p versus σ and the slope of the least squares linear regression line would be an estimate of $V_f k_f$ for the population.

(2) A plot of the extrapolated volume of distribution, V_{dext} , versus fraction free in plasma, σ , has an intercept equal to V_f and a slope equal to $(A_{TB}/A_f) V_f$, hence the ratio A_{TB}/A_f is obtained by dividing the slope by the intercept. Once V_f and A_{TB}/A_f are known, then k_f , f , and A_{TB} are readily obtained, providing there is not much variation in A_{TB}/A_f .

(3) The apparent elimination rate constant, β , is equivalent to the product of the intrinsic elimination rate constant for free drug, k_f , and the fraction of the total drug in the body which is free, symbolized by f . It should be noted that β is not equal to σk_f , unless there is absolutely no tissue binding. When β is plotted versus σ , the variables

are not separated, and the plot will have a gentle curvature. If, also, A_{TB}/A_f varies appreciably from one individual to the next, then there will be considerable scatter on the β versus σ plots. These types of plots are *not* recommended.

Since the binding of drugs to plasma proteins is extremely rapid (Keen, 1971) and the binding of some drugs to tissues is also extremely rapid (see Wagner, 1973 for diphenhydramine in the rat; and Benya and Wagner, 1975 for warfarin in the rat), one might expect the model to hold for low doses in some cases. It should be noted that if one derives equations for a similar model, but with several different types of tissues, the same general form for equation 17 is obtained, except that there are additional terms in the denominator for each type of tissue (see Wagner, 1971).

The equations derived in this article and the conclusions reached differ appreciably from some of those of Keen (1971), Coffey (1972), Krüger-Thiemer (1966 and 1968), Levy and Yacobi (1974) and Yacobi and Levy (1975). The reason for the differences is the inclusion of both tissue binding and plasma protein binding in the model and derivations in the present article, whereas previous authors cited were principally concerned only with plasma protein binding. It is hoped that this article will aid in obtaining additional pharmacokinetic information when a drug is administered by bolus intravenous injection to either a panel of subjects or patients or to a group of animals of one species. Also, it discloses some possible pitfalls in the analysis of such pharmacokinetic data.

REFERENCES

- Albert, K.S., Hallmark, M.R., Sakmar, E., Weidler, D.J., Wagner, J.G.: Pharmacokinetics of diphenhydramine in man. *J. Pharmacokin. Biopharm.* 3, 159 - 170 (1975)
- Anton, A.H., Solomon, H.M.: Drug-protein binding. *Ann. N. Y. Acad. Sci.* 226, 1 - 362 (1973)
- Benya, T.J., Wagner, J.G.: Rapid equilibration of warfarin between rat tissue and plasma. *J. Pharmacokin. Biopharm.* 3, 237 - 255 (1975)
- Bischoff, K.B., Dedrick, R.L.: Thiopental pharmacokinetics. *J. pharm. Sci.* 57, 1346 - 1351 (1968)
- Coffey, J.J.: Effects of protein binding of drugs on areas under plasma concentration-time curves. *J. pharm. Sci.* 61, 138 - 139 (1972)
- DiSanto, A.R.: A new nonlinear pharmacokinetic model with specific application to methylene blue. Ph. D. dissertation, The University of Michigan 1971
- Garrett, E.R.: Relations and conversions among variously referenced apparent volumes of distribution of the central compartment. Abstracts of papers presented at the 13th national meeting of the A. Ph. A. Academy of Pharmaceutical Sciences, Chicago, Illinois, November 5 - 9, Vol. 2, No. 2, pp. 174 - 175, 1972
- Hermann, J.J.: Physical model approach to the uptake and release of ouabain and digitoxin by human erythrocytes. Ph. D. dissertation, The University of Michigan, pp. 179, 1975
- Keen, P.: Effect of binding to plasma proteins on the distribution, activity and elimination of drugs. In: *Handbuch der experimentellen Pharmakologie* 28, 213 - 233, part 1, Berlin: Springer-Verlag 1971
- Krüger-Thiemer, E.: Solution of pharmacological problems by computers. 6. Models for the effect of protein-binding on the clearance of drugs. *Arzneimittel-Forsch.* 16, 1431 - 1442 (1966)
- Krüger-Thiemer, E.: Pharmacokinetics and dose-concentration relationships. *Proc. IIIrd Intern. Pharmacol. Meeting* 7, 63 - 113 (1968)
- Krüger-Thiemer, E., Diller, W., Bunger, P.: Pharmacokinetic models regarding protein binding of drugs. *Antimicrob. Agents Chemother.* 1965, 183 - 191 (1966)
- Levy, G., Yacobi, A.: Effect of plasma protein binding on elimination of warfarin. *J. pharm. Sci.* 63, 805 - 806 (1974)
- Martin, B.K.: Potential effect of the plasma proteins on drug distribution. *Nature* 207, 274 - 276 (1965)
- Wagner, J.G.: *Biopharmaceutics and relevant pharmacokinetics.* pp. 302 - 317. Hamilton, Illinois: Drug Intelligence Publications, Inc. 1971
- Wagner, J.G.: A modern view of pharmacokinetics. *J. Pharmacokin. Biopharm.* 1, 363 - 401 (1973)
- Wagner, J.G.: *Fundamentals of clinical pharmacokinetics.* pp. 24 - 29; 271 - 283; 381 - 382. Hamilton, Illinois: Drug Intelligence Publications, Inc. 1975
- Yacobi, A., Levy, G.: Comparative pharmacokinetics of coumarin anticoagulants. XIV: Relationship between protein binding, distribution, and elimination kinetics of warfarin in rats. *J. pharm. Sci.* 64, 1660 - 1664 (1975)

Dr. J.G. Wagner
 Upjohn Center for Clinical Pharmacology
 The University of Michigan
 Ann Arbor, Michigan 48109
 USA