noted that the parameter values reported by Kearns and Wilson were derived from a one-dog study.

No literature information regarding the pharmacokinetics of I in nephrectomized dogs is available. In this study, serum concentrations of I in nephrectomized dogs were lower than the corresponding concentrations in the normal ones. This observation suggests a greater volume of distribution for the nephrectomized dogs, which could be attributable to a reduced protein binding of I in the nephrectomized state. Reduced binding of highly bound organic acid to uremic plasma protein has been demonstrated for phenytoin, resulting in an increased volume of distribution (18). A similar mechanism may be suggested for I, which is also an organic acid with extensive protein binding (9). The reduction in binding of I to serum protein on nephrectomy is shown in Table IV for two dogs, one normal and the other nephrectomized. The protein binding was independent of concentration in the range of 5–20 μg/mL. The percentage of I unbound to serum protein in the nephrectomized dog, 6.8 ± 1.0%, was significantly greater than that in the normal dog, 1.3 ± 0.3% ($p < 0.001$). In a separate binding study of I in uremic patients (19), the fraction of free I in uremic plasma was also found to be higher than that in normal plasma (Table IV). The dogs used for this investigation were nephrectomized 3 d prior to the study and presumably were approaching the chronic uremic state.

Nephrectomy did not significantly alter the half-life of I in dogs; however, increases in both the volume of distribution and total clearance were apparent. Since <8% of the dose is excreted intact in the urine (9, 20), the lack of effect of nephrectomy on the half-life of I was anticipated. Nephrectomy induced increases in the total clearance and volume of distribution. The increased volume of distribution could result from a decreased protein binding of I in the nephrectomized state (Table IV), leaving more free drug available for distribution into the body tissue. The increase in total clearance is secondary to the increase in volume of distribution.

The effects of renal failure on the disposition of I in dogs and those of benoxaprofen in humans (16) are disparate. In dogs, the half-life of I remained unchanged, whereas the volume of distribution and total clearance increased. In humans the half-life of benoxaprofen was significantly prolonged with no distinct changes in other pharmacokinetic parameters. The disparity cautions against the extrapolation of conclusions from one drug analogue to another and from animal model to human subject.

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COMMUNICATIONS

Dynamic Method for Estimating the Extent of Plasma Protein Binding in a Dialysis Experiment

Keyphrases □ Plasma protein binding—dialysis, experimental time □ Dialysis—plasma protein binding, experimental time

To the Editor:

In a protein binding experiment one has a choice between two initial conditions: to place the drug in the plasma or in the buffer. Recent analyses (1, 2) of a linear system with a constant extent of plasma protein have revealed that it takes less time for the buffer drug concentration to reach a given limit of the equilibrium value when the drug is initially placed in the plasma rather than the buffer. This conclusion is also valid for a nonlinear system in which the number of binding sites is finite (3).

Despite the advantage of placing the drug initially in the plasma, a long experimental time may still be needed if permeation of the drug through the dialysis membrane is slow. This may cause some difficulties particularly in maintaining a constant volume in both compartments. In this report, a simple strategy is proposed to shorten the experimental time. The strategy requires two experiments: one with the drug initially placed in the buffer and the other with the drug initially placed in the plasma. The equilibrium value is derived from buffer drug concentrations measured at any given time during these two experiments. Experimental error notwithstanding, the estimate is exact provided the extent of protein binding is constant over the concentration range studied. The errors in applying the proposed method to a nonlinear situation are also considered. This is demonstrated by applying the method to a set of data simulated according to a model with saturable protein binding.

In the following discussion, we consider a dialysis experiment using a membrane with a mass transfer coefficient, M. The mass transfer coefficient is the product of the membrane permeability and the surface area. It has a dimension of volume per unit time. The volumes of the buffer in compartment 1 and the plasma in compartment 2 are V_1 and V_2, respectively. It is assumed that there is no material loss from the solution system and that there are no volume changes in either com-

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partment. Furthermore, it is assumed that the free and bound drug are in equilibrium at all times and the binding is linear such that the free fraction of the drug in the plasma is a constant, α.

The proposed approach consists of two experiments: (*a*) a given amount of drug, A_0, is initially added to the plasma compartment; (*b*) the same amount is added to the buffer compartment. When the drug is initially added to the plasma, the total plasma drug concentration is A_0/V_2, and the buffer drug concentration is zero at time zero. The buffer drug concentration at any time, t, is given as:

$$C_P(t) = C_E[1 - e^{-(1/V_1+\alpha/V_2)Mt}] \qquad \text{(Eq. 1)}$$

where subscript P is used to indicate that drug is initially added to the plasma. C_E is the buffer concentration at equilibrium and is given as:

$$C_E = \frac{A_0}{V_1 + V_2/\alpha} \qquad \text{(Eq. 2)}$$

When drug is initially added to the buffer, its concentration is $C_0 = A_0/V_1$ and the plasma concentration is zero at $t = 0$. The drug concentration in the buffer at any time t is given as:

$$C_B(t) = C_E + (C_0 - C_E)e^{-(1/V_1+\alpha/V_2)Mt} \qquad \text{(Eq. 3)}$$

where subscript B is used to indicate that all the drug is initially placed in the buffer and C_E is defined by Eq. 2.

If both C_B and C_P are measured at time t (>0), one can calculate the expected equilibrium concentration by the following expression:

$$C_E = \frac{C_0 \cdot C_P(t)}{C_0 + C_P(t) - C_B(t)} \qquad \text{(Eq. 4)}$$

Equation 4 is derived by simply combining Eqs. 1 and 3.

When the amounts of drug used for these two experiments are not identical, Eq. 4 can still be used with proper correction, because of the linearity of the system. For example, if the initial amount placed in the plasma is A'_0 instead of A_0, and the measured buffer concentration at time t is $C'_P(t)$, the corresponding buffer concentration $C_P(t)$ for an initial amount A_0 in the plasma is:

$$C_P(t) = C'_P(t) \cdot A_0/A'_0 \qquad \text{(Eq. 5)}$$

The adjusted concentration $C_P(t)$ can then be used in Eq. 4 to obtain the equilibrium concentration. Thus, Eq. 4 is a useful expression for calculating the equilibrium concentration in protein binding experiments when the free fraction remains constant over the drug concentration range studied. The basic assumptions of mass conservation and no volume shift within the time frame of the experiment should be independently verified before the application of this expression.

In the following discussion, we will consider the application of this method to situations of nonlinear binding. As an example, we consider a dialysis experiment with unit volume of plasma and buffer. The mass transfer coefficient of the dialysis membrane is 1 volume unit/unit time. There is protein in compartment 2 only. The amount of binding sites, B, in compartment 2 is 100. The dissociation constant, K, is 0.01. The differential equations governing this system are given below:

$$V_1 \frac{dC_1}{dt} = -M(C_1 - C_2) \qquad \text{(Eq. 6)}$$

$$V_2\left[1 + \frac{B \cdot K/V_2}{(K + C_2)^2}\right]\frac{dC_2}{dt} = M(C_1 - C_2) \qquad \text{(Eq. 7)}$$

where C_1 and C_2 are free drug concentrations in compartments 1 and 2, respectively. These equations are numerically solved (4) to generate C_P and C_B at $t = 0.693$ and 1.386 for different initial concentrations. The equilibrium value is then estimated from C_P and C_B using Eq. 4. The percent error of this estimate is defined as the absolute value of the difference between the estimation by Eq. 4 and the theoretical value divided by the theoretical value and multiplied by 100. The theoretical equilibrium concentration for a given initial amount of drug A_0 is:

$$C_E = \frac{-[(V_1 + V_2)K + B - A_0] + \{[(V_1 + V_2)K + B - A_0]^2 + 4A_0K(V_1 + V_2)\}^{1/2}}{2(V_1 + V_2)} \qquad \text{(Eq. 8)}$$

The error is graphically presented as a function of initial amount in Fig. 1. The errors are very small when the initial amount is small. The error starts to increase precipitously when the initial amount exceeds 90. The error peaks between 100 and 110 and eventually drops back to zero when the initial amount becomes very large. The data also indicate a significant decrease in error as the experiment is prolonged.

In addition to the errors in the equilibrium concentrations obtained using Eq. 4, the errors obtained by assuming that the buffer concentrations $C_P(t)$ (drug initially added to plasma) are estimates of the equilibrium concentrations are also presented in Fig. 1. It can be seen that a better estimate of C_E is obtained by $C_P(t)$ alone rather than by application of Eq. 4 in a region (initial amount 100–140) where nonlinear kinetics is expected to prevail. The example serves to point the dangers of the indiscriminate use of Eq. 4 in the nonlinear region.

The errors which result at both low and high initial amounts (relative to $B = 100$) can be appreciated by reference to Eq. 1. Since α, the free fraction in plasma, will vary from $\sim K/B$

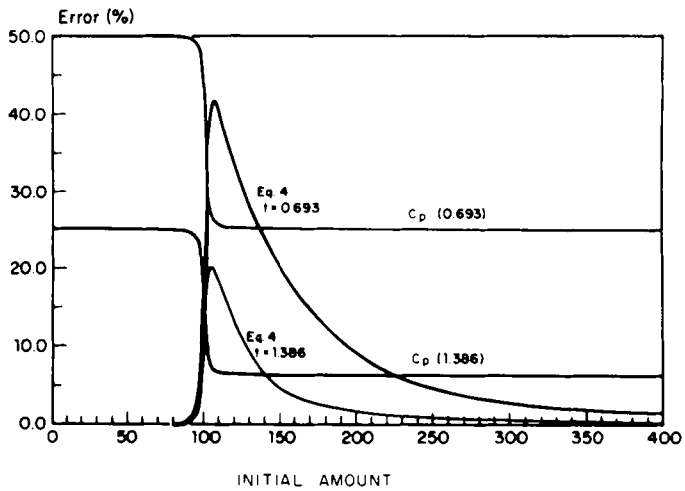

Figure 1—*Errors of estimating the equilibrium value by C_P and Eq. 4 at indicated sampling time plotted as a function of the initial amounts.*

($K \ll B$) at low initial amounts to 1 at high initial amounts, the exponent in Eq. 1 (the reciprocal of the time constant) will vary from M/V to $M/2V$ in those cases where $V_1 = V_2$. For the example considered, where the volumes are unity, the respective time constants are 1 and 0.05, and the respective half-lives are 0.693 and 0.693/2. At low initial amounts, C_P is ~0.5 C_E and 0.75 C_E at the times 0.693 and 0.693 × 2, respectively. The corresponding errors as previously defined would be 50 and 25%. At high initial amounts, C_P is ~0.75 C_E and 0.9375 C_E ($1 - 0.5^4$) C_E at the times 0.693 and 0.693 × 2, respectively. The corresponding errors are 25 and 6.25%.

A criterion for deciding whether Eq. 4 is applicable is easily established by using the equation with a few concentrations paired in time from the two experiments. Since the equilibrium concentration is time independent, the values calculated by concentrations obtained at different times should be in agreement within experimental error. Having established linearity, the free fraction α can be evaluated using Eq. 2.

Nonlinear kinetics should be suspected when a lack of consistency in the estimated equilibrium concentrations is manifested. In principle, the estimated equilibrium values calculated using Eq. 4 converge to the true equilibrium value when evaluated with concentrations obtained at later times (note how relative error changes as function of time in the nonlinear region in Fig. 1). Under such circumstances, Eq. 4 should not be employed.

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S. S. Hwang[x]
W. F. Bayne
Merck Sharp & Dohme Research Laboratories
West Point, PA 19486

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Influence of Protein Binding on the Accumulation and Depletion of Drug from the Skin

Keyphrases □ Protein binding--accumulation and depletion of drug from skin

To the Editor:

Recently the drug scopolamine has been shown to exhibit a nonlinear sorption isotherm with skin (1). The binding was deduced to be associated with the proteinaceous phase since the sorption isotherm was not significantly changed by removal of lipids (1). For drugs that bind in a saturable fashion to skin, the binding can have dramatic effects on the transport process as manifested by an increase in the diffusional lag time with a decrease in the upstream concentration (2). To elucidate the effect of reversible binding, describable by the law of mass action, on the accumulation and depletion of drug from the skin, a simple model will be considered. A fixed volume of skin will be considered to be well-stirred such that within the skin there are no gradients of free drug normal to the surface. The clearance per unit surface area of free drug from this volume is equivalent to the permeability of the drug through the skin. Sink conditions are assumed to exist, and the product of the free concentration and clearance represents the mass rate input into the body. Analysis of the simple model rather than the transport model characterized by a nonlinear diffusion equation suffices to bring forth the influence of the saturable, reversible binding on the accumulation and depletion of drug in the skin.

The dynamics of the accumulation and depletion of drug will individually be investigated as a function of the steady-state concentration in the skin. The dynamics will then be compared between the two modes at given steady-state concentrations. During the accumulation mode, steady-state concentrations will be envisioned to be established by constant fluxes applied to the skin. Upon removal of the input, the steady-state concentrations become the initial concentrations for the depletion mode. The use of the nonlinear binding to achieved prolonged delivery to the body in the depletion mode and methods to shorten accumulation rates relative to depletion rates are then discussed.

The nonlinear system will exhibit linear behavior when the binding isotherm is in the linear region or the fraction of bound drug becomes very small. Thus, a linear system serves as an extrapolative extreme for the nonlinear system. In the linear system the accumulation of drug within the skin is given by the following equation:

$$C = C_{ss}(1 - e^{-k_{el}t}) \qquad \text{(Eq. 1)}$$

$$C_{ss} = \frac{J \cdot A}{CL}$$

where C is the concentration in the skin during accumulation, C_{ss} is the steady-state concentration, J is the flux applied to the skin, A is the area to which the flux is applied, k_{el} is the elimination rate constant from the skin, CL is the clearance from the skin, and t is time. The depletion of drug from the skin once steady state has been achieved and the input removed is given by:

$$C = C_{ss}e^{-k_{el}t} \qquad \text{(Eq. 2)}$$

where C is the concentration during depletion. In Eq. 2 t is relative to the time depletion commences. The accumulation and depletion curves are symmetrical around $C = 0.5\ C_{ss}$. The symmetry of the curves is maintained regardless of the magnitude of the elimination rate constant. The time to reach 50% of the steady-state concentration in the accumulation mode and the time to fall to 50% of the steady-state concentration in the depletion mode are equal. The equality becomes apparent when the equations are rearranged to express time explicitly as a function of the ratio of the concentration at time t to the steady-state concentration. Equations 3 and 4 correspond to Eqs. 1 and 2, respectively.

$$t = (1/k_{el}) \ln \frac{1}{1 - C/C_{ss}} \qquad \text{(Eq. 3)}$$

and

$$t = (1/k_{el}) \ln \frac{C_{ss}}{C} \qquad \text{(Eq. 4)}$$

Thus, from either Eq. 3 or 4, the time at which $C/C_{ss} = 0.5$ (hereafter called t_{50}), is $(1/k_{el}) \ln 2$.

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