Pharmacokinetic Model for Simultaneous Determination of Drug Levels in Organs and Tissues

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Abstract □ An extension of the Bischoff–Dedrick pharmacokinetic model is presented. This model is derived from basic considerations of drug distribution with physiological and anatomical meaning. The Bischoff–Dedrick model can simultaneously predict drug distribution with time in blood, organs, and tissues of pharmacological interest. The parameters are applied to a 15-kg standard dog. The experimental kinetic data of thiopental in brain, plasma, liver, lean tissue, and adipose tissue in a dog are used to demonstrate the feasibility of the model. Allowable variations in the parameters are determined. In general, the kinetics of drug distribution in blood, organs, and tissues depend on the drug dosage, lipid solubility, partition coefficients, metabolism rate, excretion rate, protein binding, route of administration, sizes of organs and tissues, and blood flow rates through organs and tissues. These factors enter the kinetic model separately and explicitly so their effects on the kinetics of drug distribution can be studied to provide valuable information for optimal therapy.

Keyphrases □ Pharmacokinetic model—proposed for simultaneous determination of drug distribution with time in blood, organs, and tissues, demonstrated with thiopental in dogs □ Distribution, drug—simultaneous determination in blood, organs, and tissues, pharmacokinetic model, demonstrated with thiopental in dogs □ Thiopental—distribution in blood, organs, and tissues, pharmacokinetic model for simultaneous determination proposed, dogs

Drug concentration in blood is measured clinically because this procedure is easier and more convenient than the sampling of other tissues or fluids. Therefore, it has been used as an index of dose scheduling for therapeutics. Conventional pharmacokinetic models (1) correlate kinetic data of drug concentration in blood by using one or several exponential terms. Each exponential term in the conventional model represents a compartment; single or multiple compartments are used to curve fit the experimental kinetic data. The coefficients and rate constants in the pharmacokinetic equations are then determined from the curve-fit parameters.

Drug concentrations in blood with time depend on the drug dosage, lipid solubility, partition coefficients, metabolism rate, excretion rate, protein binding, and route of administration. Common pharmacokinetic models put all these factors into the rate constants and coefficients of the curve-fit equations. Unless the rate constants and coefficients can be expressed as explicit functions of related factors, they cannot be predicted without experimental kinetic data.

Some cardiac drugs, *e.g.*, digitalis, or ultrashort-acting barbiturates, *e.g.*, thiopental, have rather narrow margins of safety. Their kinetic distribution in blood may not provide sufficient information for adequate therapy. Digitalis kinetics in heart muscle and thiopental kinetics in brain can provide much better information for optimal therapy than the drug levels present in blood (2–4). Furthermore, knowledge of the drug distribution in blood, organs, and tissues may be necessary for providing optimal treatment. Conventional pharmacokinetic models cannot provide such information.

An entirely different approach to pharmacokinetic modeling was developed by Bischoff, Dedrick, and their coworkers (5–13), beginning in 1966. Their pharmacokinetic model simultaneously predicts the kinetics of drug distribution in blood, organs, and tissues of pharmacological interest. They successfully applied it to predict pharmacokinetics of thiopental (8), methotrexate (9, 12), and cytarabine (13). Bischoff and Dedrick (8) used the physiological parameters of a 70-kg standard male and a four-compartment model (blood, viscera, lean tissue, and adipose tissue) to simulate experimental kinetic data (2) of thiopental distribution in plasma, liver, lean tissue, and adipose tissue of the dog. They combined brain, heart, kidneys, liver, *etc.*, as a viscera compartment (8).

Since thiopental kinetics in the brain may provide valuable information for optimal therapy, an attempt was made to predict thiopental kinetics in the brain and, simultaneously, to predict thiopental kinetics in plasma, liver, lean tissue, and adipose tissue based on the physiological parameters of a 15-kg standard dog by using the Bischoff–Dedrick approach. The model can also be applied to study the pharmacokinetics of other drugs.

THEORETICAL

The data of Brodie *et al.* (2, 3) (Figs. 1 and 2), showing thiopental kinetics in plasma, liver, lean tissue, adipose tissue, and brain of the dog, were used to demonstrate the feasibility of the model. At least five body regions are defined: brain, liver, lean tissue, adipose tissue, and blood pool (blood volume in the capillary bed of any body region is included in that body region while the remaining blood is grouped together as another body region, called the blood pool). In mammals, blood from the GI area perfuses the liver. Thus, the GI tissues were added as another body region. Since there was little interest in the kinetics of thiopental in kidneys or heart, these and other organs were considered as another body region called viscera.

A diagram of blood circulation in the seven body regions is shown in Fig. 3. As blood flow carries the drug to the capillary bed where the drug diffuses into the tissue, each body region except the blood pool in Fig. 3 is further divided into two portions: the blood volume in the capillary bed and the tissue volume without blood. The physiological parameters of the model for a 15-kg standard dog are also shown in Fig. 3. A detailed discussion of the derivation of these parameters is presented in *Appendix I*.

Protein binding plays an important role in pharmacological effects and pharmacokinetics, since most drugs are bound to plasma proteins, especially to albumin and various tissue components (11). It is well recognized that not all macromolecules in blood or tissue are responsible for binding with the drug. Shen and Gibaldi (14) showed that the predicted thiopental concentrations in plasma and lean tissue, based on the total macromolecule content in organs and tissues, were substantially overestimated by the Bischoff–

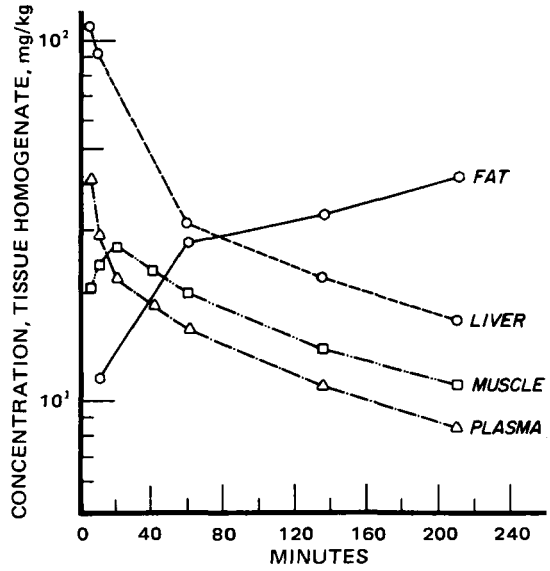

Figure 1—*Experimental thiopental concentrations in various tissues of a dog after 25 mg/kg iv (redrawn from Ref. 2).*

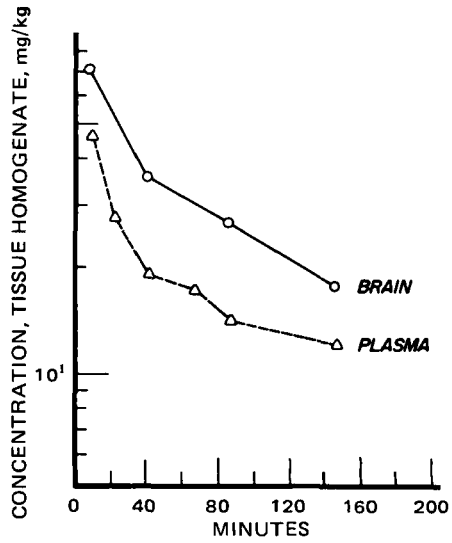

Figure 2—*Experimental thiopental concentrations in plasma and brain of a dog after 40 mg/kg iv (redrawn from Ref. 3).*

Dedrick model. Bischoff and Dedrick (7, 8) proposed the concept of effective protein fraction and applied it to their model for thiopental pharmacokinetics. In the addendum to Shen and Gibaldi's article (14), Bischoff and Dedrick favored the use of effective protein concentration rather than effective protein fraction utilizing:

$$C_T = F_W C + C_M X \qquad \text{(Eq. 1)}$$

where the total drug concentration (C_T) in blood or tissue is defined in terms of the volume fraction of water (F_W), free drug concentration (C), effective binding macromolecule concentration (C_M), and bound drug "concentration" (X, the amount of bound drug per unit of effective binding macromolecules). This approach obviates assuming unit densities, and there is no need for the two coefficients to be fractions that add up to unity.

Volume fractions of water in blood, organs, and tissues are available in the literature and in biological data handbooks. Table I lists calculated and reported values for volume fractions of water in organs and tissues of the dog. The volume fraction of water in the tissue portion of any body region can be calculated by:

$$V_{YzB}F_{W,B} + V_{YzT}F_{W,YzT} = (V_{YzB} + V_{YzT})F_{W,Yz} \qquad \text{(Eq. 2)}$$

where:

$F_{W,B}$ = volume fraction of water in blood
$F_{W,YzT}$ = volume fraction of water in tissue portion of Yz body region
$F_{W,Yz}$ = volume fraction of water in Yz body region
V_{YzB} = blood volume in Yz body region
V_{YzT} = tissue volume in Yz body region

The effective binding macromolecule concentration (C_M) in blood or tissue can be determined in terms of the fraction of bound drug (F) and the equilibrium relation between free and bound drug in blood or tissue. A detailed discussion of how effective binding macromolecule concentrations in blood and tissues were derived is presented in *Appendix II.*

The physical picture of dynamic drug distribution at any instant in any body region is as follows. The inflow drug (free and bound) mixes with the drug in the capillary bed and the mixed drug (free drug only) in the capillary bed then diffuses into the tissue. Both the changing free drug concentrations in blood water and tissue water disturb and then reestablish the equilibrium relations between free and bound drug in the capillary bed and the tissue. When a well-mixed state in the capillary bed is assumed, the outflow drug concentration (free and bound) from the body region is the same as that reestablished in the capillary bed.

For highly lipid-soluble drugs, the rate of mass transfer between blood in the capillary bed and tissue is so fast that, at any instant, the free drug concentration in tissue water effectively equals that in blood water. This condition was called the "flow-limiting condition" by Dedrick and Bischoff (7), who discussed mathematical details of the condition and found that highly lipid-soluble compounds, such as thiopental and aniline, approximate the condition.

The transient mass balance for any body region can be expressed as:

[accumulation rate of free drug and bound drug in both capillary bed and tissue portion] = [inflow rate of free drug and bound drug from blood pool and/or other body regions]

+ [drug injection rate, if any]

− [outflow rate of free drug and bound drug from body region]

− [metabolism rate and/or excretion rate of drug, if any]

(Eq. 3)

The mathematical equation of transient mass balance for body region Y (Fig. 4) is:

$$(F_{W,B}V_{YB} + F_{W,YT}V_{YT})\frac{dC_Y}{dt} + (C_{M,B}V_{YB})\frac{dX_{YB}}{dt} + (C_{M,YT}V_{YT})\frac{dX_{YT}}{dt} = Q_Y(F_{W,B}C_B + C_{M,B}X_B) - Q_Y(F_{W,B}C_Y + C_{M,B}X_{YB}) \qquad \text{(Eq. 4)}$$

where:

$$C_{M,B} = F_{Pl,B}C_{M,Pl} + (1 - F_{Pl,B})C_{M,RBC}$$

and:

C_B = free drug concentration in blood water from the blood pool (micromoles per liter)
C_Y = free drug concentration in blood water or tissue water of body region Y (micromoles per liter)
$C_{M,B}$ = effective binding macromolecule concentration in blood (kilograms per liter)
$C_{M,Pl}$ = effective binding macromolecule concentration in plasma (kilograms per liter)
$C_{M,RBC}$ = effective binding macromolecule concentration in red blood cells (kilograms per liter)
$C_{M,YT}$ = effective binding macromolecule concentration in tissue portion of body region Y (kilograms per liter)
$F_{W,B}$ = volume fraction of water in blood
$F_{W,YT}$ = volume fraction of water in tissue portion of body region Y

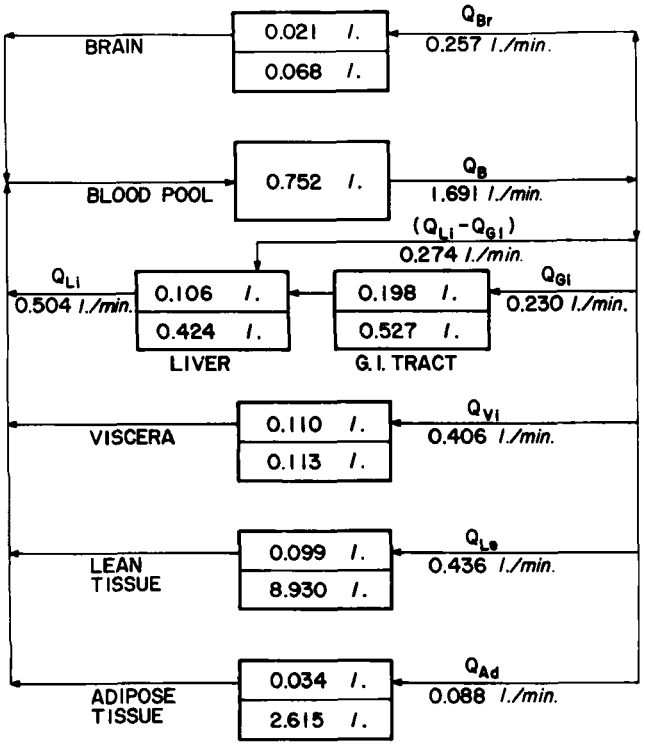

Figure 3—*Body regions and blood flow of a 15-kg dog.*

$F_{Pl,B}$ = volume fraction of plasma in blood
Q_Y = volumetric blood flow rate from body region Y (liters per minute)
t = time (minutes)
V_{YB} = blood volume in capillary bed of body region Y (liters)
V_{YT} = volume of tissue portion of body region Y (liters)
X_B = bound drug concentration (amount of bound drug per unit of effective binding macromolecules) from the blood pool (micromoles per kilogram)
X_{YB} = bound drug concentration in capillary bed of body region Y (micromoles per kilogram)
X_{YT} = bound drug concentration in tissue portion of body region Y (micromoles per kilogram)

The first term on the left-hand side of Eq. 4 represents the accumulation rate of free drug in both blood water and tissue water. The second and third terms indicate accumulation rates of bound drug in the capillary bed and in the tissue portion, respectively. The first term on the right-hand side of Eq. 4 represents the inflow rate of free drug and bound drug from the blood pool, and the second term indicates the outflow rate of free drug and bound drug from body region Y (Fig. 4).

Table I—Calculated and Reported Values for Volume Fractions of Water in Organs and Tissues of the Dog

Tissue (Yz)	$F_{W,Yz}$[a]	$F_{W,YzT}$[b]
Whole blood	0.800[c]	—
Plasma	0.930[c]	—
Red blood cells	0.640[c]	—
Whole brain	0.795[c]	0.794
Liver	0.746[c]	0.733
Intestines	0.757[c]	0.741
Heart	0.783[c]	0.775
Kidneys	0.802[c]	0.807
Viscera	0.791[d]	0.782
Lean tissue	0.776[c]	0.776
Adipose tissue	0.200[e]	0.192

[a] Reported values for volume fractions of water in various tissues. [b] Calculated values for volume fractions of water in tissue portions of various tissues. [c] P. L. Altman and D. S. Dittmer, "Biology Data Book," vol. 1, 2nd ed., Federation of American Societies for Experimental Biology, Bethesda, Md., 1972, pp. 19, 393, 394. [d] The value for volume fraction of water in viscera was calculated from heart and kidneys. [e] C. H. Best and N. B. Taylor, "The Living Body," 4th ed., Holt, Rinehart and Winston, New York, N.Y., 1966, p. 55.

Similarly, the transient mass balance was applied for each body region shown in Fig. 3. The goal was to predict thiopental kinetics in plasma. The blood was divided into plasma and red blood cells, using a hematocrit value of 45% (volume). The concept of effective binding macromolecule concentration in blood and various tissues (*Appendix II*) and the flow-limiting condition are applied to the following mathematical model.

For the blood pool:

$$F_{Pl,B} = 0.55 \qquad \text{(Eq. 5)}$$

$$C_{M,B} = F_{Pl,B}C_{M,Pl} + (1 - F_{Pl,B})C_{M,RBC} \qquad \text{(Eq. 6)}$$

The transient mass balance for the blood pool (Figs. 3 and 4) is:

$$\underbrace{(F_{W,B}V_B)\frac{dC_B}{dt}}_{\text{accumulation rate of free drug in blood pool}} + \underbrace{(C_{M,B}V_B)\frac{dX_B}{dt}}_{\text{accumulation rate of bound drug in blood pool}}$$

$$= \underbrace{Q_{Br}(F_{W,B}C_{Br} + C_{M,B}X_{BrB}) + Q_{Li}(F_{W,B}C_{Li} + C_{M,B}X_{LiB}) + Q_{Vi}(F_{W,B}C_{Vi} + C_{M,B}X_{ViB}) + Q_{Le}(F_{W,B}C_{Le} + C_{M,B}X_{LeB}) + Q_{Ad}(F_{W,B}C_{Ad} + C_{M,B}X_{AdB})}_{\text{summation of inflow rates of free and bound drug from five body regions}} + \underbrace{Mg(t)}_{\text{injection term}}$$

$$- \underbrace{Q_B(F_{W,B}C_B + C_{M,B}X_B)}_{\text{outflow rate of free and bound drug from blood pool}} \qquad \text{(Eq. 7)}$$

The particular symbols are defined under *Notation*.

The term $Mg(t)$ is the injection term, where M is the total amount of the drug injected and $g(t)$ is a normalized injection function (7, 8):

$$g(t) = 30\lambda(\lambda t)^2(1 - \lambda t)^2 \qquad \text{(Eq. 8)}$$

where λ is the reciprocal of the injection duration. The term $Mg(t)$ simulates a smooth injection and is convenient for computation (7, 8). Note that:

$$\int_0^{1/\lambda} g(t)\,dt = 1 \qquad \text{(Eq. 9)}$$

A similar mass balance for the liver (Li) gives:

$$\underbrace{(F_{W,B}V_{LiB} + F_{W,LiT}V_{LiT})\frac{dC_{Li}}{dt}}_{\text{accumulation rates of free drug in capillary bed and tissue portion of liver}} + \underbrace{(C_{M,B}V_{LiB})\frac{dX_{LiB}}{dt} + C_{M,LiT}V_{LiT})\frac{dX_{LiT}}{dt}}_{\text{accumulation rates of bound drug in capillary bed and tissue portion of liver}}$$

$$= \underbrace{(Q_{Li} - Q_{GI})(F_{W,B}C_B + C_{M,B}X_B)}_{\text{inflow rates of free and bound drug from blood pool}} + \underbrace{Q_{GI}(F_{W,B}C_{GI} + C_{M,B}X_{GIB})}_{\text{inflow rates of free and bound drug from GI area}}$$

$$- \underbrace{Q_{Li}(F_{W,B}C_{Li} + C_{M,B}X_{LiB})}_{\text{outflow rate of free and bound drug from liver}} - \underbrace{R_{Li}(C_{Li})}_{\text{metabolism rate of drug}} \qquad \text{(Eq. 10)}$$

The metabolism rate (7, 8) is written in the simple Michaelis–Menten form:

$$R_{Li}(C_{Li}) = \frac{k_{Li}C_{Li}}{k_{M,Li} + C_{Li}} \qquad \text{(Eq. 11)}$$

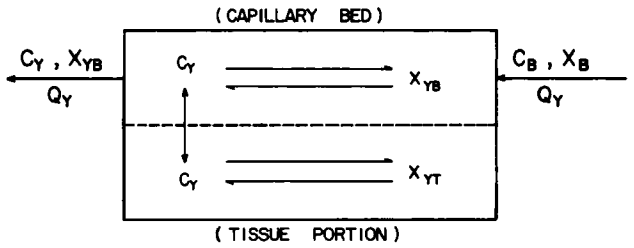

Figure 4—*Diagram of transient mass balance for the body region* Y *under the assumptions of a well-mixed state in the capillary bed and flow-limiting condition for highly lipid-soluble drugs.*

Since the metabolism mainly occurs in the liver, all metabolism of thiopental is assumed to occur in the liver. In general, similar terms could be added to other body regions if necessary. Mark (15) reported a value for the overall metabolism rate of thiopental as 15%/hr. The parameters (k_{Li} and $k_{M,Li}$) in Eq. 11 were empirically adjusted to provide values of k_{Li} = 5.68 μmoles/min and $k_{M,Li}$ = 4.0 μmoles/liter for a 15-kg standard dog. The remaining mass balances have similar forms.

For the brain (Br) region:

$$(F_{W,B}V_{BrB} + F_{W,BrT}V_{BrT})\frac{dC_{Br}}{dt} + (C_{M,B}V_{BrB})\frac{dX_{BrB}}{dt} + (C_{M,BrT}V_{BrT})\frac{dX_{BrT}}{dt} = Q_{Br}(F_{W,B}C_B + C_{M,B}X_B - F_{W,B}C_{Br} - C_{M,B}X_{BrB}) \quad \text{(Eq. 12)}$$

For the viscera (Vi) region:

$$(F_{W,B}V_{ViB} + F_{W,ViT}V_{ViT})\frac{dC_{Vi}}{dt} + (C_{M,B}V_{ViB})\frac{dX_{ViB}}{dt} + (C_{M,ViT}V_{ViT})\frac{dX_{ViT}}{dt} = Q_{Vi}(F_{W,B}C_B + C_{M,B}X_B - F_{W,B}C_{Vi} - C_{M,B}X_{ViB}) \quad \text{(Eq. 13)}$$

For the GI (GI) tissue region:

$$(F_{W,B}V_{GIB} + F_{W,GIT}V_{GIT})\frac{dC_{GI}}{dt} + (C_{M,B}V_{GIB})\frac{dX_{GIB}}{dt} + (C_{M,GIT}V_{GIT})\frac{dX_{GIT}}{dt} = Q_{GI}(F_{W,B}C_B + C_{M,B}X_B - F_{W,B}C_{GI} - C_{M,B}X_{GIB}) \quad \text{(Eq. 14)}$$

For the lean tissue (Le) region:

$$(F_{W,B}V_{LeB} + F_{W,LeT}V_{LeT})\frac{dC_{Le}}{dt} + (C_{M,B}V_{LeB})\frac{dX_{LeB}}{dt} + (C_{M,LeT}V_{LeT})\frac{dX_{LeT}}{dt} = Q_{Le}(F_{W,B}C_B + C_{M,B}X_B - F_{W,B}C_{Le} - C_{M,B}X_{LeB}) \quad \text{(Eq. 15)}$$

For the adipose tissue (Ad) region:

$$(F_{W,B}V_{AdB} + F_{W,AdT}V_{AdT})\frac{dC_{Ad}}{dt} + (C_{M,B}V_{AdB})\frac{dX_{AdB}}{dt} + (C_{M,AdT}V_{AdT})\frac{dX_{AdT}}{dt} = Q_{Ad}(F_{W,B}C_B + C_{M,B}X_B - F_{W,B}C_{Ad} - C_{M,B}X_{AdB}) \quad \text{(Eq. 16)}$$

Adipose tissue must be handled somewhat differently (*Appendix II*). It is worth noting that free drug concentrations and bound drug concentrations in all body regions (Fig. 3) are involved in Eq. 7, while the free drug concentration and bound drug concentration in the blood pool appear in all equations. Thus, each equation is not independent but interrelated to the others, constituting a set of simultaneous differential equations. There are 20 unknown variables (seven free drug concentrations in blood water or tissue water of the seven body regions and 13 bound drug concentrations in the capillary beds and tissue portions of the seven body regions) in Eqs. 7, 10, and 12–16. Seven equations can serve to solve only seven variables. Therefore, to solve the seven simultaneous equations, 13 remaining variables should be correlated to the seven selected variables by certain relationships.

Equilibrium relationships between free drug concentrations and bound drug concentrations in blood, organs, and tissues can be determined experimentally and provide the 13 relationships to solve the seven simultaneous differential equations. Actually, the equilibrium relationships between free and bound drug in the capillary beds of all body regions are the same. Hence, only seven equilibrium relations (one in the blood and six in the tissue portions of six body regions) are required.

Any equilibrium relation between free and bound drug in blood and various tissues can be determined by a general form of Langmuir adsorption (16, 17):

$$r = \sum_{i=1}^{n} \frac{N_i K_i C}{1 + K_i C} \quad \text{(Eq. 17)}$$

where:

r = moles of bound drug per mole of binding macromolecule (moles per mole)
C = free drug concentration (micromoles per liter)
N_i = average number of type i binding sites per molecule of binding macromolecule (moles per mole)
K_i = intrinsic association constant of type i binding site (liters per micromole)

Goldbaum and Smith (18) measured the binding of several barbiturates in 1% bovine serum albumin at drug levels of pharmacological interest. They correlated the observed data with the form of Langmuir adsorption for two types of binding sites:

$$r = \frac{N_1 K_1 C}{1 + K_1 C} + \frac{N_2 K_2 C}{1 + K_2 C} \quad \text{(Eq. 18)}$$

Bound drug concentration (X, micromoles of bound drug per kilogram of effective binding macromolecule) can be related to r, to be

Table II—Parameter Values for Kinetic Model of Thiopental in a 15-kg Dog

Parameter	Numerical Values
Effective binding macromolecule concentration[a], kg/liter	$C_{M,Pl}$ = 0.0146, $C_{M,RBC}$ = 0.0152, $C_{M,B}$ = 0.0149, $C_{M,BrT}$ = 0.0233, $C_{M,LiT}$ = 0.0408, $C_{M,GIT}$ = 0.0331, $C_{M,ViT}$ = 0.0409, $C_{M,LeT}$ = 0.0222, $C_{M,AdT}$ = 0.8700
Binding site constants (7, 8), μmoles/kg	B_1 = 18,400, B_2 = 305,000
Intrinsic association constant (7, 8), liters/μmole	K_1 = 0.060, K_2 = 0.000625
Lipid solubility constant (7, 8)	B_A = 100
Injection parameter[b]	M = 1420.45 μmoles, λ = 0.20 min^{-1}
Metabolism constant[b]	k_{Li} = 5.68 μmoles/min, $k_{M,Li}$ = 4.0 μmoles/liter

[a] For detailed calculation, see *Appendix II*. [b] The values were modified from Refs. 7 and 8 to suit the condition of 25 mg of thiopental/kg for a 15-kg dog.

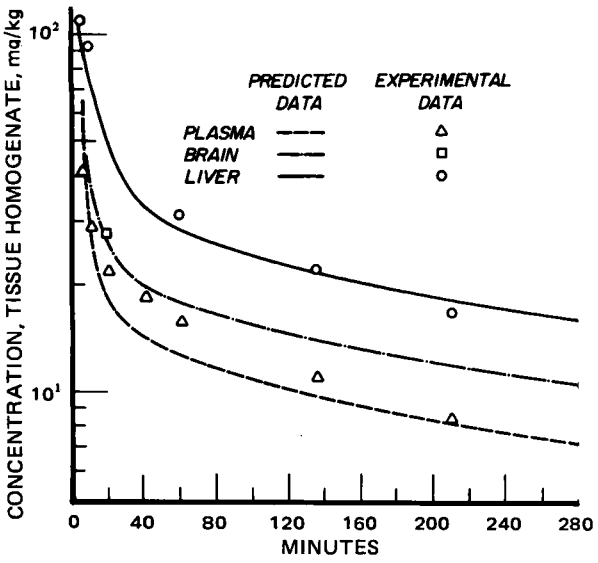

Figure 5—*Predicted and experimental thiopental concentrations in plasma, brain, and liver following 25 mg/kg iv.*

defined in terms of free drug concentration (C):

$$X = \frac{10^9}{M_P}(r) = \frac{B_1K_1C}{1+K_1C} + \frac{B_2K_2C}{1+K_2C} \qquad \text{(Eq. 19)}$$

where:

$$Bi = \frac{10^9}{M_P}(Ni)$$

where M_P = molecular weight of binding macromolecule; 68,000 was assumed for the albumin to calculate constant Bi shown in Table II.

Since no other equilibrium relationships are available, Eq. 19 with the constants in Table II was assumed to represent the equilibrium relation in blood, organs, and tissues as an approximation and was used to solve the simultaneous Eqs. 7, 10, and 12–16. This approximation is reasonable if the equilibrium relationships between free drug concentrations and bound drug concentrations in blood and various tissues of the dog have similar curve shapes to those measured in 1% bovine serum albumin by Goldbaum and Smith (18). It is assumed that the fractions of bound drug (thiopental) in blood, organs, and tissues of the dog are similar to those of the rabbit, which are available (18) and are used to calculate effective binding macromolecule concentrations (*Appendix II*). How close must they be for a reasonable approximation will be discussed later.

One may wonder whether the equilibria between free and bound drug in the body regions can be established so quickly that the equilibrium relationships can be applied to the equations of transient mass balances for the various body regions. Colowick and Womack (19), in their studies on binding of diffusible molecules by macromolecules, pointed out that the usual chemical equilibrium for the binding reaction can be reached quickly, suggesting that the equilibrium relationships between free and bound drug in blood and tissues can be applied to the equations of transient mass balances.

The term for the excretion rate is included in the general treatment (20). Since the excretion of unchanged thiopental is negligible (21), the term is omitted in this treatment.

RESULTS AND DISCUSSION

Analytical solutions of the simultaneous differential equations were not feasible because of nonlinearities caused by the binding terms. Therefore, Eqs. 7, 10, and 12–16 were solved by numerical methods (20). The numerical results for the dosage of 25 mg of thiopental/kg are presented in Figs. 5 and 6. The predicted kinetics in plasma and several organs and tissues of interest agree well with the experimental data (2).

The same mathematical model used to develop Eqs. 7, 10, and 12–16 was applied to predict the thiopental distribution in plasma and brain (3) shown in Fig. 2 by modifying some of the parameters in Table II to meet the new conditions. Because 40 mg of thiopental/kg was intravenously injected, the total amount of thiopental injected to a 15-kg dog was 2272.72 instead of 1420.45 μmoles and the injection duration was 8 min so that $\lambda = 0.125\ \text{min}^{-1}$. The predicted kinetics in plasma and brain also agree well with the experimental data (Fig. 7).

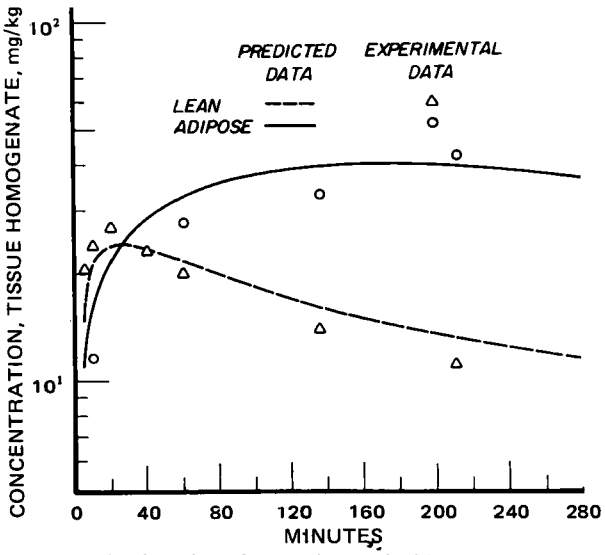

Figure 6—*Predicted and experimental thiopental concentrations in lean and adipose tissues following 25 mg/kg iv.*

Mathematical Model Testing—This mathematical model involves many parameters that must be known to certain degrees of accuracy. If the model is stable enough, some reasonable variations of the parameters should not appreciably change the results. Physiological parameters in Table I and Fig. 3 are statistical average values, which may be close to true values. Biochemical parameters in Table II are determined from experiment and are expected to deviate from true values. Because the true values for all parameters are unknown, the values for all parameters in Tables I and II and Fig. 3 were assumed to be standard values. The stability of the model was tested by comparing the numerical results from standard values for all parameters with those from a variation of ±10% of standard value for each of the following parameters:

1. ±10% variation of the volume of any body region
2. ±10% variation of the volume and the blood flow rate of any body region

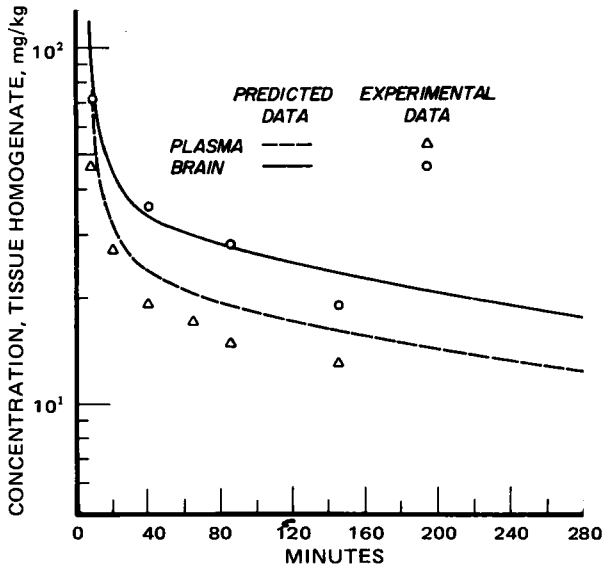

Figure 7—*Predicted and experimental thiopental concentrations in plasma and brain following 40 mg/kg iv.*

Table III—Volumes and Blood Supplies of Different Body Regions for a 15-kg Standard Dog

Tissue	Total Tissue Volume (Including Blood), liters	Tissue Volume (Excluding Blood[a]), liters	Blood Volume in Capillary Bed, liters	Blood Flow Rate, liters/min
Brain	0.089[b]	0.068	0.021[c]	0.257[d]
Liver	0.530[b]	0.424	0.106[e]	0.504[f]
Adrenals	0.002[b]	0.001	0.001[c]	0.034[d]
Kidneys	0.090[b]	0.025	0.065[c]	0.264[f]
Thyroid	0.003[b]	0.001	0.002[c]	0.027[d]
Heart	0.128[b]	0.086	0.042[c]	0.081[d]
GI	0.725[b]	0.527	0.198[e]	0.230[g]
Lean tissue	9.029[h]	8.930	0.099[f]	0.436[d]
Fatty marrow	0.480[i]	0.472	0.008[c]	0.020[d]
Fat	2.169[i]	2.143	0.026[c]	0.068[d]

[a] Calculated from total tissue volume minus blood volume in the capillary bed. [b] W. S. Spector, "Handbook of Biological Data," Saunders, Philadelphia, Pa., 1956, pp. 163, 283. [c] Calculated under the assumption that dogs and humans have the same ratio of blood volume in capillary bed to tissue volume without blood. [d] Calculated under the assumption that blood flow rate in certain tissues or organs have the same percentages of blood flow rate from the blood pool as that of standard man. [e] E. E. Selkurt, "Physiology," 2nd ed., Little, Brown, Boston, Mass., 1966, pp. 391–393. [f] D. S. Dittmer and R. M. Grebe, "Handbook of Circulation," Saunders, Philadelphia, Pa., 1959, pp. 71, 127, 129. [g] J. P. Delaney and J. Custer, *Circ. Res.*, 17, 394(1965). [h] Calculated under the assumption of 60.2% of body weight. [i] Calculated under the assumption that dogs and humans have the same percentage of body weight for the corresponding tissues.

3. ±10% variation of the value for each volume fraction of water in any body region
4. ±10% variation of the lipid solubility constant, B_A
5. ±10% variation of the metabolism constant, k_{Li}
6. ±10% variation of the binding site constants, Bi
7. ±10% and ±5% variation of the fractional data of bound drug in various tissues

The data (20) show that ±10% variation in each parameter, except fractional data of bound drug in various tissues, can be tolerated without significantly affecting the results. This finding verifies that the model is quite stable, allowing at least ±10% variations in all parameters but fractional data of bound drug where a ±5% variation is acceptable. This indicates that the values for fractions of bound drug in blood, organs, and tissues are more sensitive parameters than the others in the model and, therefore, must be determined more accurately.

Conclusions—Thiopental kinetics in brain are very important for anesthetic studies. The model not only predicts thiopental distribution in blood but also simultaneously provides thiopental kinetics in brain and other organs. Conventional pharmacokinetic models can never achieve this feature. One important feature of the model is that each equation is not independent but interrelated to the others.

One potential application is that if the model can be verified for a given drug in a species pharmacokinetically similar to humans, it can then be applied to predict human pharmacokinetics by making certain modifications in physiological and biochemical parameters. The experimentally observed drug distribution in plasma or blood can be used to compare with the corresponding kinetics predicted by the model. If they agree well, the predicted drug distribution in the other body regions that are difficult to sample may be expected to follow corresponding kinetic courses with confidence, even if they cannot be verified experimentally. Therefore, the model can provide valuable information for optimal therapeutic regimens.

Another potential application of the model is that the pharmacokinetics of some critical substances such as uric acid, creatinine, and urea in patients suffering from renal failure or of exogenous poisons in acutely intoxicated patients can be analyzed with respect to extracorporeal device treatments. In such cases, the extracorporeal device adds one more equation to the model system, and certain physiological parameters such as blood flow rates through organs and tissues must be modified. This type of analysis could lead to more optimal extracorporeal device treatment (20).

APPENDIX I: DETAILS OF PHYSIOLOGICAL PARAMETERS

The viscera body region shown in Fig. 3 includes kidneys, thyroid, heart, and adrenals; the adipose tissue body region contains fatty marrow and fat. The blood volume of the blood pool is from total blood volume (1.320 liters) minus the sum of the blood volumes of the other six body regions shown in Fig. 3. The blood flow rate from the blood pool is the sum of the blood flow rates from the other five body regions (excluding blood flow rate from the GI area) (Table III).

APPENDIX II

The effective binding macromolecule concentrations[1] (7, 14) in various tissues can be derived as follows. If there are D μmoles of drug contained in tissue homogenate of Yz body region with W liters of water (tissue water and blood water in the tissue homogenate) and P kg effective binding macromolecules out of total macromolecules in the tissue, the following relation can be established:

$$D = WC + PX \qquad \text{(Eq. A1)}$$

where C is free drug concentration in blood water or tissue water (micromoles per liter), and X is bound drug concentration (the amount of bound drug per unit of effective binding macromolecules) in the tissue (micromoles per kilogram). The fraction of bound drug in the tissue homogenate, F, is defined as $F = PX/D$. Substituting this into Eq. A1 gives:

$$D = (1-F)D + PX \qquad \text{(Eq. A2)}$$

When Eq. A2 is divided by the total volume of the tissue homogenate, it becomes:

$$C_T = (1-F)C_T + C_{M,Yz}X \qquad \text{(Eq. A3)}$$

where C_T is the total drug concentration, and $C_{M,Yz}$ is the effective binding macromolecule concentration of Yz tissue homogenate. This relationship can be further simplified:

$$C_{M,Yz} = \frac{FC_T}{X} \qquad \text{(Eq. A4)}$$

Substituting Eq. 19 into Eq. A4 gives:

$$C_{M,Yz} = FC_T \Big/ \left(\frac{B_1K_1C}{1+K_1C} + \frac{B_2K_2C}{1+K_2C}\right) \qquad \text{(Eq. A5)}$$

The effective binding macromolecule concentration in any tissue homogenate (Table IV) can be calculated by using Eq. A5 if the fraction of bound drug (F) in a certain total drug concentration (C_T) and the equilibrium relationship between the free drug concentration and bound drug concentration in the tissue homogenate are known.

The effective binding macromolecule concentration in the tissue portion (Table IV) can be calculated by the following equations,

[1] K. B. Bischoff, School of Chemical Engineering, Cornell University, Ithaca, NY 14850, and R. L. Dedrick, Biomedical Engineering and Instrumentation Branch, National Institutes of Health, Bethesda, MD 20014, personal communication

Table IV—Binding Parameters of Thiopental in Various Organs and Tissues of the Rabbit

Parameter	Liver	Heart	Kidneys	Brain	Lean Tissue	Red Blood Cells	Plasma, Undiluted	Viscera[a]	GI Tissue[a]
F^b	0.66	0.62	0.53	0.50	0.45	0.40	0.85	0.58	—
$C_{M,Yz}{}^c$	0.0356	0.0313	0.0235	0.0213	0.0221	0.0152	0.0146	0.0281	0.0281
$C_{M,YzT}{}^c$	0.0408	0.0393	0.0459	0.0233	0.0222	—	—	0.0409	0.0331

[a] Data shown were calculated by the method discussed in *Appendix II.* [b] Fractions of bound drug in organs and tissues except plasma were determined from tissue homogenate diluted to five volumes (18). [c] $C_{M,Yz}$ and $C_{M,YzT}$ are the effective binding macromolecule concentrations, which have been multiplied by five (dilution factor), in tissue homogenate and in the tissue portion, respectively. The values shown were calculated by the method discussed in *Appendix II.*

taking the liver as an example:

$$C_{M,B} = F_{Pl,B}C_{M,Pl} + (1 - F_{Pl,B})C_{M,RBC} \qquad \text{(Eq. A6)}$$

$$\frac{V_{LiB}}{V_{LiB}+V_{LiT}}C_{M,B} + \frac{V_{LiT}}{V_{LiB}+V_{LiT}}C_{M,LiT} = C_{M,Li} \qquad \text{(Eq. A7)}$$

The viscera body region consists of kidneys, heart, thyroid, and adrenals. Since the volume fraction of the thyroid and adrenals in the viscera is negligible and their fractional bound thiopental data are not available, effective binding macromolecule concentrations in kidneys (Ki) and heart (He) (Table IV) were used to calculate the effective binding macromolecule concentration in the viscera by the following equation:

$$C_{M,Vi} = \frac{V_{Ki}C_{M,Ki} + V_{He}C_{M,He}}{V_{Ki} + V_{He}} \qquad \text{(Eq. A8)}$$

Fractional bound drug data are not available for GI tissue, so its effective binding macromolecule concentration was not calculated. Therefore, the value of the effective binding macromolecule concentration in the viscera was assigned to the GI tissue. The effective binding macromolecule concentration in the GI tissue portion was then determined.

The tissue binding in adipose tissue[1] is somewhat different from that in other tissues and must be handled differently. The equilibrium relationship between free and bound drug in the capillary bed of adipose tissue is the same as Eq. 19:

$$X_{AdB} = C_{Ad}\left(\frac{B_1K_1}{1+K_1C_{Ad}} + \frac{B_2K_2}{1+K_2C_{Ad}}\right) \qquad \text{(Eq. A9)}$$

Total drug concentration in the capillary bed of adipose tissue, $C_{T,AdB}$, is defined as:

$$C_{T,AdB} = F_{W,B}C_{Ad} + C_{M,B}X_{AdB} \qquad \text{(Eq. A10)}$$

Substituting Eq. A9 into Eq. A10 gives:

$$C_{T,AdB} = C_{Ad}\left[F_{W,B} + C_{M,B}\left(\frac{B_1K_1}{1+K_1C_{Ad}} + \frac{B_2K_2}{1+K_2C_{Ad}}\right)\right] \qquad \text{(Eq. A11)}$$

The tissue "binding" by adipose tissue is primarily the lipid solubility for which a simple linear Henry's law solubility relationship can be used (7, 8). Thus, drug concentration in adipose tissue (X_{AdT}) is proportional to free drug concentration (C_{Ad}) in tissue water:

$$X_{AdT} = B_AC_{Ad} \qquad \text{(Eq. A12)}$$

Total drug concentration in adipose tissue portion ($C_{T,AdT}$) is defined as:

$$C_{T,AdT} = F_{W,AdT}C_{Ad} + C_{M,AdT}X_{AdT} \qquad \text{(Eq. A13)}$$

When Eq. A12 is substituted into Eq. A13, Eq. A13 becomes:

$$C_{T,AdT} = C_{Ad}(F_{W,AdT} + C_{M,AdT}B_A) \qquad \text{(Eq. A14)}$$

The partition coefficient of adipose tissue (K_{Ad}) is defined as the ratio of total drug concentration in adipose tissue to that in its blood:

$$K_{Ad} = \frac{C_{T,AdT}}{C_{T,AdB}} = \frac{F_{W,AdT} + C_{M,AdT}B_A}{F_{W,B} + C_{M,B}\left(\frac{B_1K_1}{1+K_1C_{Ad}} + \frac{B_2K_2}{1+K_2C_{Ad}}\right)} \qquad \text{(Eq. A15)}$$

Since[1] $K_{Ad} = 15$ and $B_A = 100$, at $C_{Ad} = 100$ (μmoles/liter), $C_{M,AdT}$, which can be solved by Eq. A15, is 0.870.

NOTATION

C_{Yz} = free drug concentration in tissue water or blood water of Yz body region (micromoles per liter)
$C_{M,Yz}$ = effective binding macromolecule concentration in Yz body region (kilograms per liter)
$C_{M,YzT}$ = effective binding macromolecule concentration in tissue portion of Yz body region (kilograms per liter)
$F_{W,B}$ = volume fraction of water in blood
$F_{W,YzT}$ = volume fraction of water in tissue portion of Yz body region
Q_B = blood flow rate from blood pool (liters per minute)
Q_{Yz} = blood flow rate from Yz body region (liters per minute)
V_{YzB} = blood volume within capillary bed of Yz body region (liters)
V_{YzT} = volume of tissue portion of Yz body region (liters)
X_{YzB} = bound drug concentration (amount of bound drug per unit of effective binding macromolecules) in capillary bed of Yz body region (micromoles per kilogram)
X_{YzT} = bound drug concentration (amount of bound drug per unit of effective binding macromolecules) in tissue portion of Yz body region (micromoles per kilogram)
Yz = various body regions and tissues

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Hypocholesterolemic Agents IV: Inhibition of β-Hydroxy-β-methylglutaryl Coenzyme A Reductase by Arylalkenyl and Arylepoxy Hydrogen Succinates and Glutarates

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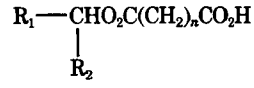
Abstract □ Two series of half acid esters of succinic and glutaric acids were synthesized and assayed for inhibition of rat liver β-hydroxy-β-methylglutaryl coenzyme A reductase. Irreversible inhibition was studied by incorporation of a potential alkylating group (the epoxide function) into the side chain of the alcohol portion of the half acid esters. Incorporation of a terminal olefin function into the side chain of the alcohol portion of the half acid esters provided a group that could form a charge–transfer complex. Neither irreversible inhibition nor formation of a charge–transfer complex was indicated from these studies; however, the two series of half acid esters exhibited reversible inhibition.

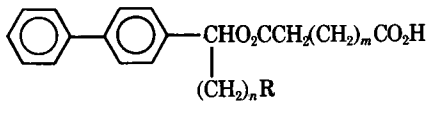
Keyphrases □ Hypocholesterolemic agents, potential—series of arylalkenyl and arylepoxy hydrogen succinates and glutarates, synthesis, effect on β-hydroxy-β-methylglutaryl coenzyme A reductase □ Enzymes—β-hydroxy-β-methylglutaryl coenzyme A reductase, inhibition by series of arylalkenyl and arylepoxy hydrogen succinates and glutarates □ Succinates, arylalkenyl and arylepoxy—substituted series, synthesis, effect on β-hydroxy-β-methylglutaryl coenzyme A reductase □ Glutarates, arylalkenyl and arylepoxy—substituted series, synthesis, effect on β-hydroxy-β-methylglutaryl coenzyme A reductase

Previous reports (1, 2) presented an approach to the design of inhibitors of the enzyme β-hydroxy-β-methylglutaryl coenzyme A reductase as potential hypocholesterolemic agents. These studies led to the development of reversible inhibitors (I) of yeast and rat liver β-hydroxy-β-methylglutaryl coenzyme A reductase. Structure–activity relationships indicated that maximum activity was observed when R_1 = biphenylyl, R_2 = *n*-butyl, and n = 1–4.

This study describes the synthesis and assay of potential irreversible inhibitors (II) of β-hydroxy-β-methylglutaryl coenzyme A reductase based upon I.



I: $n = 0$–6



II: R = —CH(O)CH$_2$, n = 1–3, m = 1 or 2

III: R = —CH=CH$_2$, n = 1–3, m = 1 or 2

The epoxide group was selected as the potential alkylating group on the basis of its relatively small size and its reactivity with nucleophiles.

The arylalkenylcarbinols (V), which were precursors of the arylepoxycarbinols (VI) used in the synthesis of the epoxy acid esters (II), were also esterified to give the corresponding alkenyl acid esters (III). Not only were these alkenyl acid esters readily available from a synthetic point of view, but they also could provide useful information when assayed as potential reversible inhibitors. The possibility exists that the terminal olefinic bond found in these alkenyl acid esters might act as an electron donor in the formation of a charge–transfer complex. Alkenes are well known for acting as donors in the formation of a charge–transfer complex because of their electron-rich π-clouds (3).

EXPERIMENTAL[1]

Chemistry—The previously described method (1) of preparing the arylalkylcarbinols was used with only slight modifications. Alkenyl bromides were utilized and the resulting arylalkenylcarbi-

[1] Melting points were determined with a Thomas-Hoover capillary melting-point apparatus and are uncorrected. Elemental analyses were carried out by Mr. Paul E. Marecki, Department of Pharmaceutical Chemistry, and by Galbraith Laboratories, Knoxville, Tenn. IR spectra were determined using a Perkin-Elmer model 237 spectrophotometer. NMR spectra were determined using a Perkin-Elmer model R-24 spectrometer or a Varian Associates A-60 spectrometer in deuterochloroform, using tetramethylsilane as the internal reference.