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Keyphrases

Coumarin anticoagulants
Bishydroxycoumarin (BHC)—distribution, elimination
Biologic half-life—dose dependency
Liver perfusion, isolated—experimental technique
Pharmacokinetics—BHC distribution, elimination

Comparative Pharmacokinetics of Coumarin Anticoagulants IV

Application of a Three-Compartmental Model to the Analysis of the Dose-Dependent Kinetics of Bishydroxycoumarin Elimination

By R. NAGASHIMA, G. LEVY*, and R. A. O'REILLY†

It has been reported that the elimination of bishydroxycoumarin (BHC) in man shows unusual dose-dependent characteristics. Recent studies with isolated perfused rat livers have shown that the liver:plasma distribution ratio of BHC increases at high doses (resulting in more rapid elimination of the drug) and that drug-metabolizing activity is apparently inhibited at high BHC levels (resulting in decreased elimination of the drug). A mathematical model based on a three-compartmental open system consisting of a plasma compartment, a rapidly accessible drug-metabolizing compartment, and a more slowly accessible compartment has been developed for a detailed pharmacokinetic analysis of BHC elimination in man. This analysis shows that the increase in the plasma half-life of BHC in man with increasing dose is due primarily to a decrease in the activity of the drug-elimination process and not to dose-dependent distribution effects. The three-compartmental model presented here has two unique attributes; the first compartment (following intravenous administration) is equated to the plasma volume rather than combining plasma and the so-called well perfused tissues into a single compartment, and an apparent volume of distribution which remains constant throughout the terminal elimination phase (β-phase) has been defined.

THERE ARE NOW several examples of drugs which are eliminated exponentially from the plasma (following absorption and initial distribution) but where the apparent first-order rate constant for drug elimination decreases with increasing dose (1). One of the earliest and best documented examples is the elimination of bishydroxycoumarin (BHC) in man (2, 3) and monkeys (4). Recent investigations with isolated perfused rat livers have shown that the distribution of BHC into the liver (*i.e.*, the site of biotransformation) increases with increasing drug concentration but that BHC apparently inhibits its own biotransformation when high concentrations of the drug are present in the liver (5). Physical-chemical studies of the interaction between BHC and plasma proteins

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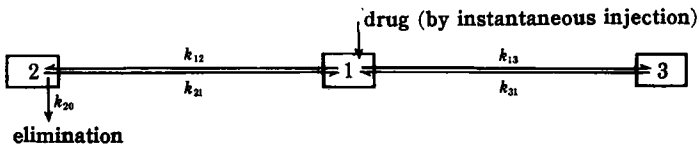
have shown also that the protein binding of BHC *increases* with increasing drug concentrations up to a point (6). Accordingly, it appears that the dose dependency of BHC elimination in man may be due to both distribution effects and apparent self-inhibition of biotransformation. Plasma concentration data for BHC following intravenous administration of this drug at three different doses, utilizing a very intensive blood sampling schedule, have been obtained so that a detailed pharmacokinetic analysis of BHC elimination in man could be carried out. For this purpose, a three-compartmental open pharmacokinetic model has been developed. This model has the unique but realistic feature of representing the actual plasma volume as the first compartment. As such, it overcomes the definite limitations of the single- and two-compartmental models which are apparent in the analysis of the experimental data obtained following BHC administration.

THEORETICAL

When a drug is administered intravenously it is first distributed in the plasma and from there to other tissues. These other tissues can be classified conveniently as well perfused and poorly perfused, respectively (see *Reference 8* for a review of the literature on this subject). The usual two-compartmental pharmacokinetic models combine the plasma and the well perfused tissues into a single compartment. The basis of the three-compartmental model to be presented here is the concept that drug distribution between plasma and well perfused tissues is not instantaneous, and that it is realistic therefore to treat these as two separate compartments. This is particularly appropriate in the case of certain highly plasma protein bound or macromolecular substances. The concept of the plasma as the first compartment is justified when values of dose/$(P + A + B)$ are essentially equal to the actual plasma volume. A pharmacokinetic analysis of this type usually requires very intensive blood sampling; where technical difficulties make this impossible, the use of simpler mathematical models becomes appropriate.

The three-compartmental open model may be depicted by Scheme I.



Scheme I

METHODS

Experimental Details—BHC was administered intravenously to a normal male subject (age 51 years, body weight 70 kg.) as the disodium salt in a concentration of 5 mg./ml. of solution. Doses of 150, 286, and 600 mg. BHC were administered in separate experiments. The drug was injected slowly over a period of 5 to 30 min., depending on the size of the dose. The instant when the injection had been completed was taken as zero time. Blood samples were collected at the times indicated in the figures. The concentration of BHC in the plasma was determined by the method of Axelrod *et al.* (7). Additional details have been described in a previous report (2).

Data Analysis—The plasma concentrations (C_p) of BHC were plotted as a function of time on semilogarithmic paper. Using back-extrapolation procedures, each of the curves was resolved into three exponential components. The data could therefore be described by the equation:

$$C_p = P \exp(-\pi t) + A \exp(-\alpha t) + B \exp(-\beta t) \quad \text{(Eq. 1)}$$

where P, A, and B represent intercepts on the ordinate at zero time and $-\pi/2.3$, $-\alpha/2.3$, and $-\beta/2.3$ are the slopes of the respective exponential segments. By definition, $\pi > \alpha > \beta$.

Compartment 1 represents the plasma volume Compartment 2 is designated as the rapidly accessible compartment [*i.e.*, the well perfused tissues such as the liver, kidneys, and viscera (9)], and Compartment 3 is designated as the slowly accessible compartment (*i.e.*, the poorly perfused tissues such as muscle, bone, and fat depots). Drug elimination is assumed to take place in Compartment 2. The processes designated by arrows and rate constants are conceived to be apparent first-order, the rate constants being defined with respect to the amount (rather than concentration) of the drug in the respective compartment. The classification of body tissues on the basis of rate of accessibility or perfusion does not imply that prefusion rate is necessarily a limiting step in the transfer of the drug from one real or hypothetical compartment to the other. It should be noted that the model presented here applies only to drugs such as bishydroxycoumarin which are eliminated by biotransformation and not by renal excretion from Compartment 1.

Evaluation of Rate Constants—As shown in *Appendix I*, the fraction of the administered dose of a drug present in each of the three compartments at time t is given by:

$$X_1/X_0 = C_1 \exp(-b_1 t) + C_2 \exp(-b_2 t) + C_3 \exp(-b_3 t) \quad \text{(Eq. 2)}$$

$$X_2/X_0 = C_1' \exp(-b_1 t) + C_2' \exp(-b_2 t) + C_3' \exp(-b_3 t) \quad \text{(Eq. 3)}$$

$$X_3/X_0 = C_1'' \exp(-b_1 t) + C_2'' \exp(-b_2 t) + C_3'' \exp(-b_3 t) \quad \text{(Eq. 4)}$$

where X_1, X_2, and X_3 are the amounts of the drug in Compartments 1, 2, and 3, respectively, X_0 is the dose (the initial amount of drug in Compartment 1) and C's and b's are time-independent constants. It is important to note that the right sides of the three equations differ only with respect to the C's. In particular, Eq. 2, which represents the fraction of the dose in the plasma, is equivalent to Eq. 1 which describes plasma concentration of the drug as a function of time. This is so, since

$$\frac{X_1}{X_0} = \frac{C_p}{C_p^0}$$

and, by definition,

$$C_p^0 = P + A + B$$

Therefore, the following relationships apply:

$$\left.\begin{aligned} b_1 &= \pi & C_1 &= P/C_p^0 \\ b_2 &= \alpha & C_2 &= A/C_p^0 \\ b_3 &= \beta & C_3 &= B/C_p^0 \end{aligned}\right\} \quad \text{(Step I)}$$

π, α, and β, as well as P, A, and B are obtained directly from the plasma concentration data.

Let

$$K_1 = k_{12} + k_{13}$$
$$K_2 = k_{21} + k_{20}$$
$$K_3 = k_{31}$$

Then, as shown in *Appendix I*,

$$\left.\begin{aligned} K_2 &= (R + Q)/2 \\ K_3 &= (R - Q)/2 \end{aligned}\right\} \quad \text{(Step III)}$$

and

where

$$\left.\begin{aligned} R &= C_1(b_3 - b_1) + C_2(b_3 - b_2) + b_1 + b_2 \\ Q &= [\{C_1(b_3 - b_1) + C_2(b_3 - b_2) + b_1 - b_2\}^2 + 4C_2(b_2 - b_1)(b_3 - b_2)]^{1/2} \end{aligned}\right\} \quad \text{(Step II)}$$

Let

$$\left.\begin{aligned} G_1 &= b_1 + b_2 + b_3 \\ G_2 &= b_1 b_2 + b_2 b_3 + b_3 b_1 \\ G_3 &= b_1 b_2 b_3 \end{aligned}\right\} \quad \text{(Step IV)}$$

Then,

$$K_1 = G_1 - K_2 - K_3 \quad \text{(Step V)}$$

Knowing the values for K_1, K_2, and K_3, the individual rate constants k_{12}, k_{21}, k_{20}, and k_{13} can be determined from:

$$\left.\begin{aligned} k_{13} &= \frac{G_3 - K_3(G_2 - K_1K_3 - K_2K_3)}{K_3(K_3 - K_2)} \\ k_{12} &= K_1 - k_{13} \\ k_{21} &= (K_1K_2 + K_2K_3 + K_3K_1 - G_2 - k_{13}K_3)/k_{12} \\ k_{20} &= K_2 - k_{21} \end{aligned}\right\} \quad \text{(Step VI)}$$

Recall that $k_{31} = K_3$. It should be noted that k_{20} represents the intrinsic drug-metabolizing activity of the body and excludes factors which affect the distribution of the drug in the three compartments. The latter factors are represented by k_{12}, k_{21}, k_{13}, and k_{31}.

The sequence of calculations by which the rate constants are determined have been outlined for the convenience of the reader in terms of 6 separate steps. In Step I, b and C values are calculated. These are then used in Step II to calculate R and Q. This permits determination of K_2 and K_3 in Step III. Determination of G values in Step IV permits the determination of K_1 in Step V. All terms necessary for the determination of k values in Step VI are now available. While these calculations can be carried out manually, the use of a computer will result in considerable saving of time.

An Analysis of the Relative Effect of Distribution and Biotransformation on the Slope of the Terminal Linear Portion (β-Phase) of a Log Plasma Concentration versus Time Plot—In the course of an experiment, the curves expressed by Eqs. 2, 3, and 4 eventually reduce to:

$$X_1/X_0 = C_3 \exp(-\beta t) \quad \text{(Eq. 5)}$$

$$X_2/X_0 = C_3' \exp(-\beta t) \quad \text{(Eq. 6)}$$

$$X_3/X_0 = C_3'' \exp(-\beta t) \quad \text{(Eq. 7)}$$

where $\beta = b_3$ as defined previously. Therefore, if the fractions of the dose in each of the compartments are plotted semilogarithmically against time, curves are obtained which become linear in the terminal portion (β-phase). Let the total amount of the drug in the body at any given time be X_B so that

$$X_B = X_1 + X_2 + X_3$$

Also let

$$f_1 = X_1/X_B$$
$$f_2 = X_2/X_B$$
$$f_3 = X_3/X_B$$

The fractions f_1, f_2, and f_3, therefore, become constant during the β-phase and may be expressed on the basis of Eqs. 5, 6, and 7 as:

$$f_1 = C_3/(C_3 + C_3' + C_3'')$$
$$f_2 = C_3'/(C_3 + C_3' + C_3'')$$
$$f_3 = C_3''/(C_3 + C_3' + C_3'')$$

Substituting for C_3, C_3', and C_3'' the equations presented in *Appendix I* yield:

$$f_1 = (K_2 - b_3)(K_3 - b_3)/Z \quad \text{(Eq. 8)}$$

$$f_2 = k_{12}(K_3 - b_3)/Z \quad \text{(Eq. 9)}$$

$$f_3 = k_{13}(K_2 - b_3)/Z \quad \text{(Eq. 10)}$$

where

$$Z = (K_2 - b_3)(K_3 - b_3) + k_{12}(K_3 - b_3) + k_{13}(K_2 - b_3).$$

The total amount of the drug in the body at a given time, X_B, may be expressed as:

$$X_B = X_1/f_1 \quad \text{(Eq. 11)}$$

and

$$X_1 = V_p \cdot C_p \quad \text{(Eq. 12)}$$

where V_p is the actual plasma volume and C_p is drug concentration in the plasma. Since during the β-phase,

$$C_p = B \exp(-\beta t) \quad \text{(Eq. 13)}$$

Eqs. 11, 12, and 13 may be combined to yield:

$$X_B = \frac{V_p}{f_1} B \exp(-\beta t) \quad \text{(Eq. 14).}$$

At $t = 0$, Eq. 14 reduces to:

$$X_B^0 = \frac{V_p}{f_1} \cdot B \quad \text{(Eq. 15)}$$

which upon substitution into Eq. 14 yields:

$$X_B = X_B^0 \exp(-\beta t) \quad \text{(Eq. 16)}$$

It should be noted that X_B^0 is not the dose X_0 but a quantity defined by Eq. 15. Equation 16 shows that the apparent first-order rate constant for the elimination of drug from the body as a whole is identical to the apparent rate constant obtained from plasma concentration data in the terminal phase of the elimination process. Therefore, the value $0.693/\beta$ is justifiably called "body half-life" or "biologic half-life." This reasoning leads to the following expression for drug elimination from the body during the β-phase:

$$\frac{dX_B}{dt} = -\beta X_B \quad \text{(Eq. 17)}$$

In terms of the three-compartmental model, where drug elimination takes place only in Compartment 2,

$$\frac{dX_B}{dt} = -k_{20} \cdot X_2$$

and, as shown previously,

$$X_2 = f_2 \cdot X_B.$$

Hence,

$$\frac{dX_B}{dt} = -k_{20} \cdot f_2 \cdot X_B \quad \text{(Eq. 18)}$$

Equating Eqs. 17 and 18, yields:

$$\beta = f_2 \cdot k_{20} \quad \text{(Eq. 19)}$$

Equation 19 has important implications in that it shows that β is a function of the distribution of the drug in the body (specifically, the fraction f_2 of the total amount of the drug which is in the drug-metabolizing compartment) and of the intrinsic drug metabolizing activity as represented by the rate constant k_{20}. This conclusion has been verified experimentally in this laboratory (5).

The derivations presented here lead to a concept of an apparent volume of distribution which has considerable practical advantages. Since, during the β-phase of drug elimination, f_1 is a time-independent constant, the following definition may be introduced

$$V_d^* = \frac{V_p}{f_1} \quad \text{(Eq. 20)}$$

The amount of drug in the body at any time during the β-phase, can therefore be calculated from the expression:

$$X_B = V_d^* \cdot B \exp(-\beta t) \quad \text{(Eq. 21)}$$

which is based on Eq. 14. This volume of distribution is totally different from that defined by Riggs (10) and adopted by Wagner and Northam (11), and Riegelman *et al.* (12). Their volume of distribution multiplied by plasma concentration equals the amount of drug in the body only at the one instant when the amount of the drug in the "peripheral" compartment reaches its maximum and cannot be used to determine the amount of drug in the body from drug concentrations in the plasma at any other time (15).

To calculate V_d^* from Eq. 20, V_p may be obtained from the body weight [plasma volume is about 4.3% of body weight (13)], and f_1 is calculated from Eq. 8 which consists of terms the determination of which is described in the first part of this paper. The use of a digital computer for these calculations is desirable since any rounding-off of values during the large number of individual calculations can lead to large errors in the final results. In the present study, the original data introduced into the computer program were expressed in two or three significant figures, and the computer carried out all subsequent calculations to a maximum of nine significant figures. The final answers printed out by the computer were then rounded off to three figures.

Determination of the Apparent Volumes of the Rapidly and Slowly Accessible Hypothetical Compartments—Defining

$$V_d^* = V_p + V_2 + V_3$$

where V_2 and V_3 are the apparent volumes of Compartment 2 and Compartment 3 with respect to a specific drug, and

$$C_p V_d^* = \frac{C_p V_p}{f_1} = \frac{C_p V_2}{f_2} = \frac{C_p V_3}{f_3} \quad \text{(Eq. 22)}$$

then

$$\frac{V_p}{f_1} = \frac{V_2}{f_2}$$

and

$$V_2 = V_p \cdot \frac{f_2}{f_1}$$

Similarly,

$$V_3 = V_p \cdot \frac{f_3}{f_1}$$

The values of V_2 and V_3 can therefore be determined from V_p and Eqs. 8 to 10.

RESULTS AND DISCUSSION

Primary Analysis of the BHC Plasma Concentration Data—The plasma concentrations of BHC following intravenous administration of 150, 286, and 600 mg. are listed in Table I and plotted in Fig. 1. Secondary concentration maxima were noted 20 to 24 hr. following injection of the two higher doses. The reason for the occurrence of these maxima, which reflect apparently a redistribution of the drug, is not clear at present. The pharmacokinetic analysis of the data was limited

TABLE I—CONCENTRATIONS OF BISHYDROXYCOUMARIN (BHC) IN THE PLASMA FOLLOWING INTRAVENOUS ADMINISTRATION OF DIFFERENT DOSES OF BHC TO A NORMAL HUMAN SUBJECT[a]

Dose, mg.					
150		286		600	
Time, hr.	Concn., mg./l.	Time, hr.	Concn., mg/l.	Time, hr.	Concn., mg./l.
0.17	36.2	0.17	60.5	0.17	120
0.33	34.0	0.51	49.8	0.33	111
0.50	27.0	1	37.2	0.50	104
0.67	23.2	2.83	27.8	0.67	96
1	20.8	5	22.2	1	93
1.5	17.8	7.25	18.1	1.5	86
2	16.5	11	14.1	2	86
3	13.9	13	12.6	3	74
4	12.0	21	8.4	5	70
6	8.7	24.3	8.3	7.5	65
7.7	7.7	36	5.6	18	44
18	3.2	48	2.6	21	39
23.3	2.4			27	34
				32	38
				42	29
				48	27
				68.5	17
				92	9
				121	4

[a] Male, 70 kg. body weight, 51 years old.

therefore to the first 20 to 24-hr. portion of the curves and these data are presented in a more expanded form in Fig. 2. The results of the resolution of these data into three exponential components are shown in Figs. 2 and 3. The values of the intercepts P, A, and B, and of π, α, and β for the three segments are listed in Table II. It is evident that π and α are dose independent while β decreases with increasing dose. The values for dose/$(P + A + B)$ listed in Table II, and particularly the value for the lowest dose, are in good agreement with the plasma volume, *i.e.*, about 3 l. for a 70-kg. man (13). It is to be expected that the long injection times for the two higher doses will have had a pronounced distorting effect on P and a somewhat smaller effect on A. Since π is equivalent to a $t_{1/2}$ of about 20 min. while the time required for the injection of the 600-mg. dose was 30 min., a negative time shift was carried out in the case of the 286-mg. and 600-mg. data, using the π, α, and β values listed in Table II but setting dose/$(P + A + B)$ = 3.2 l. The corrected P, A, and B values obtained by this procedure are listed in Table III. The zero time shift required for these corrections was −19 min. in each instance.

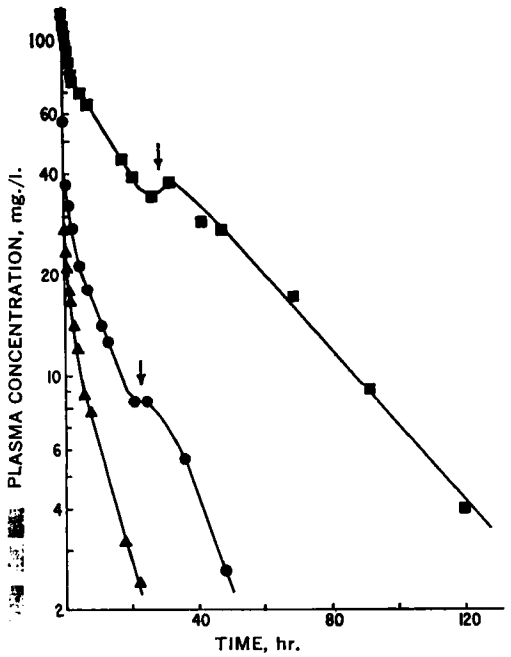


Fig. 1—Concentration of bishydroxycoumarin in the plasma as a function of time after intravenous administration of 150 mg., ▲; 286 mg., ●; and 600 mg., ■, respectively, to a normal human subject. The arrows indicate secondary concentration maxima observed 1 day after drug administration.

Determination of Pharmacokinetic Constants Based on a Three-Compartmental Open System—Using the mathematical procedures described in the Theoretical section, the apparent first-order rate constants k_{20}, k_{12}, k_{21}, k_{13}, and k_{31} were computed from the values for π, α, and β (Table II) and from the corrected values for P, A, and B (Table III) by means of an IBM 7044 digital computer. The results of these computations are listed in Table IV. Essentially similar values for all rate constants except for k_{20} were obtained at the three doses, but k_{20} decreased with increasing dose. The distribution of BHC in the three compartments as a function of dose is listed in Table V which shows the fraction of the total amount of the drug in each of the three compartments. It can be noted that f_2, the fraction of the total amount of drug present in the compartment in which the drug is metabolized, shows no consistent change with dose. This leads to the significant conclusion, based on the relationship represented by Eq. 19, that the dose dependence in the elimination of BHC as reflected by a decrease in β with increasing dose is due to a decrease in the intrinsic drug-metabolizing activity and not to distribution effects.

Finally, a comparison of the product of f_2 and k_{20} as obtained from the lengthy and complex calculations described in the *Theoretical* section with

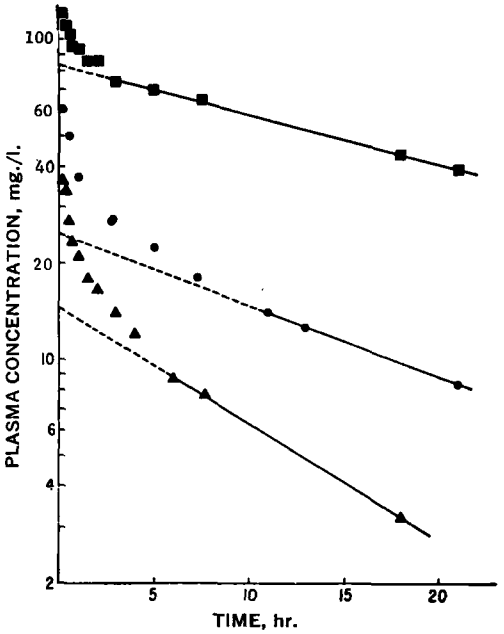


Fig. 2—The data from Fig. 1 presented on an expanded scale over 24 hr. after drug administration. The slope of the linear segment of each curve is designated as $-\beta/2.3$ and the intercept on the ordinate at zero time is designated as B. *Symbols as in Fig. 1.*

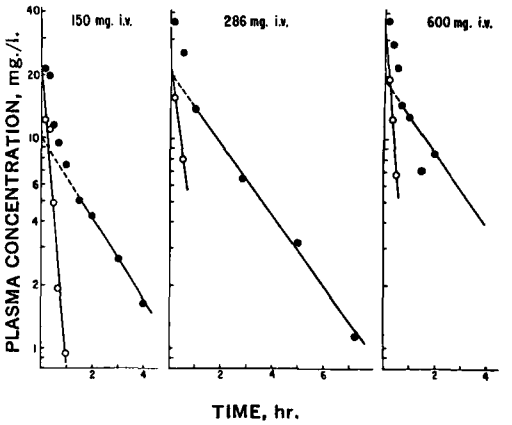


Fig. 3—Plots of residuals obtained by subtracting from the initial plasma concentrations of bishydroxycoumarin shown in Fig. 2, the respective theoretical concentration values represented by the stippled lines (solid symbols), and by subtracting from the initial data points represented by the solid symbols in this figure the theoretical values represented by the stippled lines (open symbols). The slope of the line fitted to the solid symbols is designated as $-\alpha/2.3$ and the intercept at zero time is designated as A. *The slope of the line fitted to the open symbols is designated as $-\pi/2.3$ and the intercept at zero time is* P.

TABLE II—PHARMACOKINETIC PARAMETERS OBTAINED BY RESOLVING THE BHC PLASMA CONCENTRATION DATA INTO THREE COMPONENTS

Parameter[a]	Dose, mg. 150	286	600	Units
P	22	22	34	mg./l.
A	10	22	19	mg./l.
B	14.5	24.5	83	mg./l.
Dose/$(P + A + B)$	3.2	4.2	4.4	l.
π	3.5	2.3[b]	3.5	hr.$^{-1}$
α	0.46	0.41	0.41	hr.$^{-1}$
β	0.085	0.051	0.036	hr.$^{-1}$

[a] $C_p = P \exp(-\pi t) + A \exp(-\alpha t) + B \exp(-\beta t)$ where C_p is the plasma concentration of BHC at time t. The other symbols are defined in the text and in Figs. 2 and 3. [b] Based on 2 data points only.

β values obtained by simply fitting a straight line to the terminal log plasma concentration values provides a direct check on the mathematical derivations and the accuracy of the calculations. The $f_2 \cdot k_{20}$ values calculated from the data in Tables IV and V are 0.085, 0.052, and 0.036 hr.$^{-1}$ for the 150-, 286-, and 600-mg. doses, respectively. These agree perfectly with the β values listed in Table II.

TABLE III—THE VALUES FOR P, A, AND B WHEN CORRECTED FOR VARIABLE DURATION OF THE INTRAVENOUS INJECTION[a]

	Dose, mg. 150	286	600
P	22	40	81
A	10	23	22
B	14.5	25	84
Zero time shift, min.	−0	−19	−19

[a] Expressed as mg./l. The corrections are based on an initial value for dose/$(P + A + B)$ of 3.2 l.

APPENDIX I

The transfer and elimination processes in the three-compartmental open system presented in the *Theoretical* section may be described as follows:

$$\frac{dX_1}{dt} = -K_1X_1 + k_{21}X_2 + k_{31}X_3$$

$$\frac{dX_2}{dt} = -K_2X_2 + k_{12}X_1$$

$$\frac{dX_3}{dt} = -K_3X_3 + k_{13}X_1$$

In these expressions, X is the amount of drug in the designated compartment, the numerical subscripts for X define the compartment, the respective k's are defined in the model presented in the *Theoretical* section, and

$$K_1 = k_{12} + k_{13} \qquad \text{(Eq. 1}a\text{)}$$

TABLE IV—APPARENT FIRST-ORDER RATE CONSTANTS CHARACTERIZING THE THREE-COMPARTMENTAL OPEN SYSTEM FOR THE DISTRIBUTION AND ELIMINATION OF BHC[a]

Rate Constants, hr.$^{-1}$	Dose, mg. 150	286	600
k_{20}	0.295	0.235	0.11
k_{12}	1.56	0.91	1.44
k_{21}	1.67	1.13	1.93
k_{13}	0.22	0.26	0.14
k_{31}	0.30	0.23	0.32

[a] See Scheme I in the *Theoretical* section.

TABLE V—DISTRIBUTION PATTERN OF BHC IN THE TERMINAL ELIMINATION PHASE (β-PHASE)

Compartment[a]	Dose, mg. 150	286	600
1, plasma (f_1)	0.35	0.31	0.455
2, rapidly accessible, drug eliminating (f_2)	0.29	0.22	0.33
3, slowly accessible (f_3)	0.36	0.47	0.215

[a] f_1, f_2, and f_3 are the fractions of the total amount of drug in the body present in Compartments 1, 2, and 3, respectively, and were calculated from Eqs. 8, 9, and 10.

$$K_2 = k_{20} + k_{21} \qquad \text{(Eq. 2}a\text{)}$$

$$K_3 = k_{31} \qquad \text{(Eq. 3}a\text{)}$$

In order to solve the above simultaneous differential equations by using Laplace transforms, it is convenient to set up the following determinant:[1]

$$\Delta = \begin{vmatrix} s + K_1 & -k_{21} & -k_{31} \\ -k_{12} & s + K_2 & 0 \\ -k_{13} & 0 & s + K_3 \end{vmatrix} = (s + b_1)(s + b_2)(s + b_3) \qquad \text{(Eq. 4}a\text{)}$$

where $-b_1$, $-b_2$, and $-b_3$ are the real roots of the equation, $\Delta = 0$. With the initial condition that $X_1 = X_0 =$ dose when $t = 0$, the fractions of the dose in each of the three compartments are:

$$X_1/X_0 = C_1 \exp(-b_1 t) + C_2 \exp(-b_2 t) + C_3 \exp(-b_3 t)$$

$$X_2/X_0 = C_1' \exp(-b_1 t) + C_2' \exp(-b_2 t) + C_3' \exp(-b_3 t)$$

$$X_3/X_0 = C_1'' \exp(-b_1 t) + C_2'' \exp(-b_2 t) + C_3'' \exp(-b_3 t)$$

where the C values (with various superscripts and subscripts) are constants. These may be evaluated on the basis of the following relationships:

$$C_j = \left[\frac{\Delta_{1,1}}{\Delta'}\right] s = -b_j$$

$$C_j' = \left[\frac{-\Delta_{1,2}}{\Delta'}\right] s = -b_j$$

$$C_j'' = \left[\frac{\Delta_{1,3}}{\Delta'}\right] s = -b_j$$

where the subscript j represents either subscript 1, 2, or 3, $\Delta_{1,i}$ is the determinant obtained from Δ by suppressing the first row and the ith column,[2] and Δ' is $d\Delta/ds$ of the right hand of Eq. 4a.[3]

$$C_1 = \frac{(K_2 - b_1)(K_3 - b_1)}{(b_2 - b_1)(b_3 - b_1)};$$

$$C_2 = \frac{(K_2 - b_2)(K_3 - b_2)}{(b_1 - b_2)(b_3 - b_2)};$$

$$C_3 = \frac{(K_2 - b_3)(K_3 - b_3)}{(b_1 - b_3)(b_2 - b_3)}$$

$$C_1' = \frac{k_{12}(K_3 - b_1)}{(b_2 - b_1)(b_3 - b_1)};$$

$$C_2' = \frac{k_{12}(K_3 - b_2)}{(b_1 - b_2)(b_3 - b_2)};$$

$$C_3' = \frac{k_{12}(K_2 - b_3)}{(b_1 - b_3)(b_2 - b_3)}$$

$$C_1'' = \frac{k_{13}(K_2 - b_1)}{(b_2 - b_1)(b_3 - b_1)};$$

$$C_2'' = \frac{k_{13}(K_2 - b_2)}{(b_1 - b_2)(b_3 - b_2)};$$

$$C_3'' = \frac{k_{13}(K_2 - b_3)}{(b_1 - b_3)(b_2 - b_3)}$$

The next step is to develop equations from which the values for k_{20}, k_{12}, k_{21}, k_{13}, and k_{31} may be determined from the known values for b_1, b_2, b_3, C_1, C_2, and C_3. Rearrangement and expansion of the equation for C_1 yields:

$$C_1(b_2 - b_1)(b_3 - b_1) = K_2K_3 - b_1(K_2 + K_3) + b_1^2 \qquad \text{(Eq. 5}a\text{)}$$

Similarly for C_2:

$$C_2(b_1 - b_2)(b_3 - b_2) = K_2K_3 - b_2(K_2 + K_3) + b_2^2 \qquad \text{(Eq. 6}a\text{)}$$

Defining

$$R = K_2 + K_3 \qquad \text{(Eq. 7}a\text{)}$$

$$q = K_2K_3 \qquad \text{(Eq. 8}a\text{)}$$

and solving for R and q from the simultaneous Eqs. 5a and 6a, yields:

$$R = C_1(b_3 - b_1) + C_2(b_3 - b_2) + b_1 + b_2 \qquad \text{(Eq. 9}a\text{)}$$

$$q = b_2[C_1(b_3 - b_1) + C_2(b_3 - b_2) + b_1] + C_2(b_1 - b_2)(b_3 - b_2) \qquad \text{(Eq. 10}a\text{)}$$

From Eqs. 7a and 8a, and on the basis of $K_2 > K_3$ (see *Appendix II*),

$$K_2 = (R + \sqrt{R^2 - 4q})/2 \qquad \text{(Eq. 11}a\text{)}$$

$$K_3 = (R - \sqrt{R^2 - 4q})/2 \qquad \text{(Eq. 12}a\text{)}$$

Substituting Eqs. 9a and 10a for R and q in Eqs. 11a and 12a, and simplifying, gives the following

[1] The reader may find it helpful to refer to *Reference 14* for examples of similar derivations based on other models.

[2] For example,

$$\Delta_{1,2} = \begin{vmatrix} -k_{12} & 0 \\ -k_{13} & s + K_3 \end{vmatrix} = -k_{12}(s + K_3).$$

[3] Specifically,

$$\Delta' = (s + b_2)(s + b_3) + (s + b_1)(s + b_3) + (s + b_1)(s + b_2).$$

TABLE VI—CONSTANTS DESCRIBING THE DISTRIBUTION AND ELIMINATION OF BHC ASSUMING THAT $K_3 > K_2$ (SEE *Appendix II*)

Constants[a]	Dose, mg. 150	286	600
k_{20}	0.152	0.0845	0.118
k_{12}	0.457	0.416	0.215
k_{21}	0.145	0.140	0.209
k_{13}	1.32	0.751	1.37
k_{31}	1.97	1.37	2.04
f_1	0.26	0.25	0.41
f_2	0.56	0.60	0.31
f_3	0.18	0.14	0.28

[a] Units of k = hr.$^{-1}$; f has no units.

equations for K_2 and K_3:

$$K_2 = (R + Q)/2$$

$$K_3 = (R - Q)/2$$

where $Q = \sqrt{R^2 - 4q}$. These are the equations for K_2 and K_3 presented in the Theoretical section.

Expanding both sides of Eq. 4*a* and identification of equivalent terms yields:

$$b_1 + b_2 + b_3 = K_1 + K_2 + K_3 \quad \text{(Eq. 13}a\text{)}$$

$$b_1b_2 + b_2b_3 + b_3b_1 = K_1K_2 + K_2K_3 + K_3K_1 - k_{12}k_{21} - k_{13}k_{31} \quad \text{(Eq. 14}a\text{)}$$

$$b_1b_2b_3 = K_1K_2K_3 - k_{12}k_{21}K_3 - k_{13}k_{21}K_2 \quad \text{(Eq. 15}a\text{)}$$

K_1 can now be evaluated from Eq. 13*a*, since K_2 and K_3 are already known. The values for $k_{12}k_{21}$ and $k_{13}k_{31}$ are obtained from the two simultaneous equations 14*a* and 15*a*. Since $k_{31} = K_3$ and has already been determined from Eq. 12*a*, k_{13} can be determined from the value of $k_{13}k_{31}$. The value of k_{12}, obtained from Eq. 1*a*, is used to solve for k_{21} from $k_{12}k_{21}$. Finally, k_{20} is obtained from Eq. 2*a*, since k_{21} and K_2 are now known.

APPENDIX II

The data treatment leading to Eqs. 11*a* and 12*a* assumes that $K_2 > K_3$ (*i.e.*, $k_{20} + k_{21} > k_{31}$). The opposite assumption ($K_3 > K_2$) resulted in $k_{13} > k_{12}$, which is inconsistent with the proposed model. However, this latter possibility (which means that drug metabolism occurs in the slowly accessible compartment) cannot be excluded solely on the basis of the plasma concentration data although it is unlikely on the basis of physiologic considerations. A similar uncertainty occurs in the use of two-compartment models although this has apparently been disregarded so far in the pharmacokinetic literature.

When the experimental data were analyzed on the basis of the assumption that $K_3 > K_2$, k_{20} did not change consistently with dose but k_{12} decreased with increasing dose (Table VI). It is interesting that the k_{12} values are quite similar to the k_{21} values, and k_{13} values to k_{31} values, in the model assuming drug metabolism in the rapidly accessible compartment (Table V). This is not the case where $K_3 > K_2$ (Table VI). Preliminary evidence based on the relationship between the plasma concentration and pharmacologic activity of BHC in man is consistent with the assumption that $K_2 > K_3$. However, a definitive conclusion concerning the two possibilities will require determinations of BHC biotransformation rates at various drug concentrations in human liver preparations.

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Keyphrases

Coumarin anticoagulants

Pharmacokinetics—bishydroxycoumarin elimination

Dos-dependence—bishydroxycoumarin elimination

Plasma concentration—bishydroxycoumarin

Kinetic equations—three-compartmental model