Anal.—Calcd. for $C_{12}H_{13}ClN_2 \cdot HCl$: C, 56.0; H, 5.49; N, 10.9. Found: C, 56.0; H, 5.47; N, 10.7.

2,2-Dimethyl-8-chloro-1,2,3,4-tetrahydro-β-carbolinium Iodide (XXVII)—A mixture of 0.8 g. (3.9 mmoles) of 8-chlorotetrahydro-β-carboline (XIV), 0.16 g. (4 mmoles) of sodium amide, and 50 ml. of toluene was refluxed with stirring for 4 hr. After cooling 0.57 g. (4 mmoles) of methyl iodide was added, and the mixture was first stirred at room temperature for 1 hr. then refluxed for 1 hr. The solvent was evaporated *in vacuo* and the residue washed with water; yield, 1.0 g., m.p. 188–208°. Another washing with benzene gave 700 mg. (48%), m.p. 240–242°. Recrystallization from ethanol gave 250 mg., m.p. 238–239°. The product gave a positive test for iodine (5). $\lambda_{max.}$ (KBr): 2.93, 3.15, 3.32, 3.42, 3.51 (NH, CH); 6.18, 6.28, 6.45, 6.79, 6.85, 6.95 μ (C=C, CH_2).

Anal.—Calcd. for $C_{13}H_{16}ClIN_2$: C, 43.1; H, 4.45, N, 7.73. Found: C, 43.3; H, 4.42; N, 7.62.

Assay—Mitochondrial monoamine oxidase from beef liver was isolated and purified as previously described (1). All the stock solutions of the hydrochloride salts of inhibitors except XI·HCl were prepared in water. Compounds VI, VIII, and XII were dissolved in 0.01 *N* HCl, Compounds V and XI in dimethyl sulfoxide, and Compound XIV in 25% aqueous dimethyl sulfoxide. Incubation was carried out with tryptamine-2-^{14}C hydrochloride according to the previously described procedure (1).

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Comparative Pharmacokinetics of Coumarin Anticoagulants VI: Effect of Plasma Protein Binding on the Distribution and Elimination of Bishydroxycoumarin by Rats

GERHARD LEVY and RENPEI NAGASHIMA*

Abstract □ The partitioning of bishydroxycoumarin (BHC) from rat plasma to an organic solvent phase decreases with increasing drug concentration to a minimum value and then increases as the concentration is further increased. The same type of profile is observed in the partitioning of BHC from rat plasma to the liver, both *in vitro* and *in vivo*. The elimination of large doses (60 mg./kg.) of BHC in the rat is much more rapid than the elimination of smaller (2-20 mg./kg.) doses. A plot of the elimination rate constant of BHC as a function of dose yields a curve which is similar to the partitioning profiles of BHC from plasma to liver and from plasma to organic solvent. The minimum concentration ratio, liver:plasma, in a perfused liver system and in intact animals, and the minimum *in vivo* elimination rate constant, occur at the same plasma concentration of BHC. These results reflect the unusual concentration dependence of the plasma protein binding of this drug. They demonstrate the pronounced effect of protein binding on the distribution of BHC, and the effect of distribution on the elimination of this drug.

Keyphrases □ Coumarin anticoagulants—pharmacokinetics □ Bishydroxycoumarin (BHC)—distribution, elimination □ Elimination rates—dose relationship □ Plasma protein binding—BHC distribution, elimination

Bishydroxycoumarin (BHC) is a widely used anticoagulant that is extensively bound to plasma albumin and is eliminated from the body almost exclusively by biotransformation in the liver (1). Its unusual pharmacokinetic characteristics in man (2), which are also evident in the rhesus monkey (3), have stimulated numerous investigations (reviewed in *Reference* 4). Because BHC also shows an unusual concentration dependence in its binding to plasma protein (4), this drug is particularly suitable as a model to determine the effect of plasma protein binding on the distribution of a drug in the body, and to assess the effect of changes in distribution on the kinetics of its elimination. Accordingly, the plasma protein binding, distribution, and kinetics of elimination of BHC have been studied over a wide concentration range and will be described here.

EXPERIMENTAL

The extractability of BHC from rat plasma (4), the isolated rat liver perfusion system (1), and the method of determination of BHC in rat plasma (4) have been described in previous papers in this series. About 60 mg./kg. body weight of BHC was administered intravenously or intraperitoneally to male Sprague-Dawley rats weighing 240-600 g. A 5-mg./kg. dose was administered 1 week later. All other experimental conditions were as described previously (3), except that 6 mg./kg. vitamin K_1 (Aqua Mephyton, Merck Sharp and Dohme, West Point, Pa.) was administered intraperitoneally in all experiments immediately before injection of BHC. The apparent volume of distribution (V_d), the biologic half-life

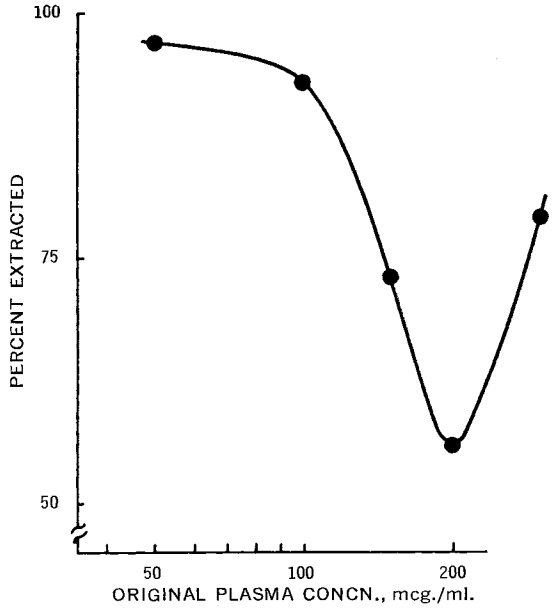

Figure 1—*Extractability of BHC from rat plasma (pH < 1) into n-heptane as a function of concentration before extraction (average of 2 or 3 determinations).*

($t_{0.5}$), and the apparent first-order rate constant for the elimination of BHC from the plasma ($k_{app.}$) were determined (3) from plots of log BHC concentration in plasma *versus* time over approximately 13 hr.

RESULTS

The distribution of BHC between acidified (pH < 1) rat plasma and *n*-heptane is concentration-dependent (Fig. 1). The drug is almost completely extracted into the organic phase at low (< 100 mcg./ml.) concentrations, but is retained in the plasma to a much greater extent as the concentration increases to 200 mcg./ml. The extractability then increases as the BHC concentration increases beyond 200 mcg./ml.

Figure 2 shows the concentration ratio of BHC between the isolated perfused rat liver and plasma as a function of the BHC concentration in the plasma phase. Since the blood used to perfuse an isolated liver must be diluted somewhat, the actual concentrations of BHC in the liver perfusate were multiplied by 1.7 to correct for this dilution (1). The concentration ratio *versus* plasma concentration profile in Fig. 2 is qualitatively similar to the distribution profile shown in Fig. 1.

The liver to plasma concentration ratio of BHC as a function of plasma concentration in intact rats (Fig. 3) was determined from the data published by Christensen (5). Shown also in Fig. 3 are estimates of the apparent first-order rate constant for BHC elimination calculated from pairs of consecutive plasma concentration values, plotted against the respective midpoint concentrations of BHC in the plasma. It may be noted that the minimum liver:plasma concentration ratio of BHC in intact rats (Fig. 3) occurred at the same plasma concentration (≃ 40 mcg./ml.) as in the isolated liver perfusion system (Fig. 2).

The administration of high (> 50 mg./kg.) doses of BHC to intact rats presented considerable difficulty due to acute toxic effects of this drug. Because of the high mortality (5/7) from intravenous injections, the drug was administered intraperitoneally in some cases. Figure 4 shows the time course of BHC concentrations in the plasma of a rat following intraperitoneal injection of 60-mg./kg. and 5-mg./kg. doses, respectively, of BHC. The plasma concentrations decreased exponentially with time and there was no evidence of a distribution or absorption phase after 1.5 hr., the time of the first blood sample. Essentially the same pattern was observed with the other rats. The rate constant for the decline of BHC concentrations in the plasma and the apparent volume of distribution were considerably larger with the high doses than with the lower doses, the average ratio being 1.7 in each case (Table I).

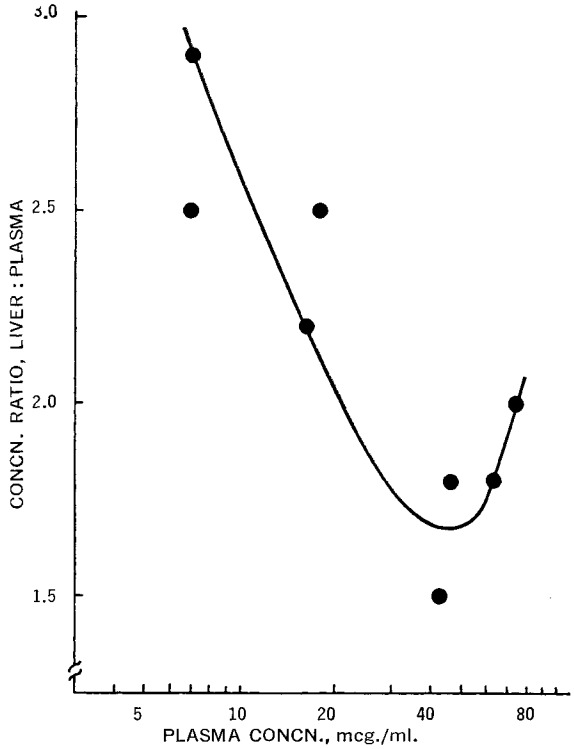

Figure 2—*Partition ratios of BHC between liver and plasma as a function of plasma concentration of the drug in isolated rat liver perfusion experiments. The plasma concentration at the midpoint (2 hr.) of perfusion was multiplied by a factor of 1.7 to correct for the dilution of the rat plasma and thus to obtain estimates of concentrations comparable to those in intact animals.*

Apparent first-order elimination rate constants for BHC obtained in this and a previous study (3) are plotted as a function of dose in Fig. 5. The lowest rate constant was observed at a dose of 5 mg./kg. The average initial (extrapolated to zero time) plasma concentration of BHC at this dose is 47 mcg./ml., which is in essentially

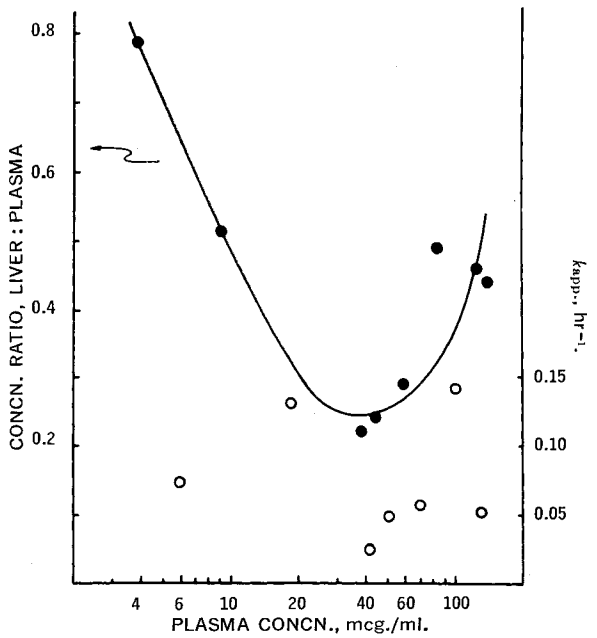

Figure 3—*Partition ratios of BHC between liver and plasma, and apparent first-order rate constants for BHC elimination from plasma ($k_{app.}$) as functions of plasma concentration of the drug after intravenous administration of approximately 27 mg. BHC/kg. to intact rats. Calculated from the data in* Reference 5.

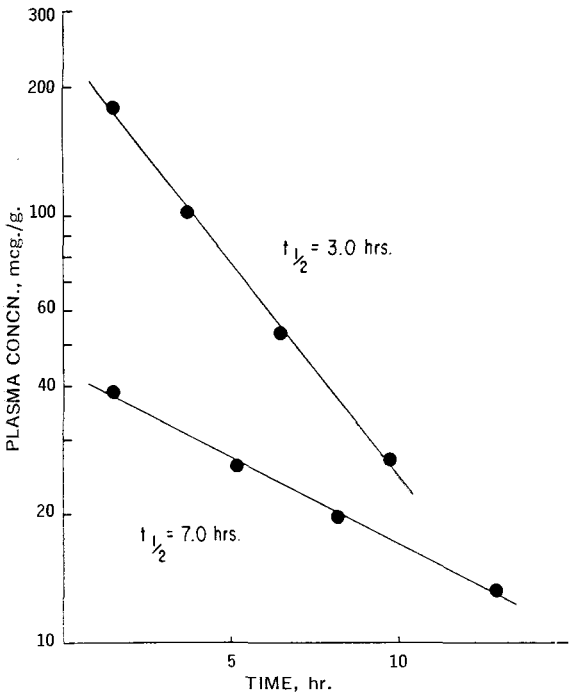

Figure 4—*Plasma concentration of BHC as a function of time in a rat (No. 5) which received high (60 mg./kg.) and low (5 mg./kg.) intraperitoneal doses of BHC.*

the same concentration range as that at which the minimum liver:plasma distribution of BHC occurs both in intact rats (Fig. 3) and in perfused rat liver systems (Fig. 2).

DISCUSSION

The results of this study are striking evidence of the effect of plasma protein binding on drug distribution, and of distribution on the kinetics of elimination of a drug from the body. These effects are of course particularly pronounced in the case of a drug such as BHC, which is not excreted as such except in minute quantities (5), and which is highly bound to plasma proteins (2). The partition data (Fig. 1) cannot be compared directly to the results of the other experiments since the plasma had to be acidified to afford measurable extractability. They do however illustrate clearly the concentration dependence of the binding of BHC to plasma albumin. This is probably due to a so-called cooperative effect, with the albumin molecule apparently undergoing a reversible configurational alteration during the binding process and thereby yielding additional binding sites (4). Consequently there is more pronounced drug-albumin interaction with increasing drug concentration up to the point where a limit in the binding capacity is approached. A further increase in drug concentration results in a progressive decrease in the protein binding of the drug.

Table I—Pharmacokinetic Constants for Bishydroxycoumarin Elimination from Plasma in Rats[a]

Animal No.	Body Wt.,g.	Dose, mg./kg.	$t_{0.5}$, hr.	$k_{app.}$, hr.$^{-1}$[b]	V_d, ml./kg.[c]
3	530	56.6[d]	4.6	0.15	142
	496	5.0[d]	6.8	0.10	100
4	253	60.0[d]	3.5	0.20	240
	269	5.0[e]	5.3	0.13	109
5	244	60.0[e]	3.0	0.23	178
	243	5.0[e]	7.0	0.10	112

[a] Sprague-Dawley males. Vitamin K_1 6 mg./kg. was administered intraperitoneally immediately prior to BHC. [b] Apparent first-order rate constant for elimination of BHC from plasma. [c] Apparent volume of distribution. [d] Intravenous injection. [e] Intraperitoneal injection.

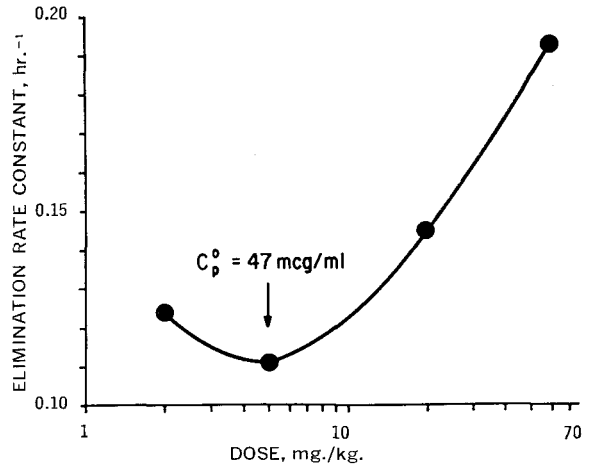

Figure 5—*Plot of apparent first-order rate constant for BHC elimination in rats as a function of dose. Based on data from Table I and* Reference **3**.

A similar concentration dependence is evident in the distribution of BHC between liver and plasma, both *in vitro* (Fig. 2) and *in vivo* (Fig. 3). The higher concentration ratio *in vitro* is due to the necessary dilution of the blood used for the liver perfusion (0.7 ml. Ringer solution/ml. plasma). It should be noted that the concentration of BHC in the liver was up to three times higher than in the perfusion fluid in the *in vitro* experiments. This may be due to the interaction of BHC with proteins and/or lipids in the liver, but it could also reflect the existence of an active transport process. There is in fact evidence for energy-dependent translocation of BHC into rat liver mitochondria (6). Regardless of the mechanism(s) involved, changes in the extent of binding of BHC to plasma albumin will affect the activity of BHC in the plasma and therefore the liver:plasma distribution ratio of the drug. However, it is unrealistic in most cases to incorporate an expression for concentration-dependent plasma protein binding of drugs in pharmacokinetic models, while disregarding the interaction of such drugs with proteins and other molecules in other tissues. The distribution of a drug in the body is a function of both types of interaction, among other factors.

A previous study in this series (1) has shown that the apparent first-order rate constant for BHC elimination in an isolated perfused rat liver system is a function of the BHC concentration at the site of biotransformation (*i.e.*, the concentration in the liver rather than in the blood). The apparent rate constant for BHC elimination ($k_{app.}$) is therefore the product of the "true" rate constant (k) and the fraction (F) of the total amount of drug in the system which is actually in the liver:

$$k_{app.} = k \cdot F \qquad \text{(Eq. 1)}$$

A subsequent pharmacokinetic analysis of the dose-dependent kinetics of BHC elimination in man (7) yielded an analogous expression:

$$\beta = f_2 \cdot k_{20} \qquad \text{(Eq. 2)}$$

where $-\beta/2.3$ is the slope of the last exponential segment of a multiexponential plot of log BHC concentration in the plasma *versus* time, f_2 is the fraction of the total amount of drug which is in the drug-metabolizing compartment, and k_{20} is the rate constant for drug metabolism in the compartment where this process takes place. The effect of distribution on the biotransformation kinetics of a drug is therefore readily apparent.

The *in vivo* elimination kinetics of BHC in rats after administration of 5- and 60-mg./kg. doses were determined in order to extend previously determined pharmacokinetic data (3) over a wider dose range. It was necessary to co-administer vitamin K_1 in order to prevent fatal hemorrhages after the high doses of BHC. This vitamin does not affect the distribution and metabolism of BHC (8). The two doses of BHC were administered to the same animals and blood samples were drawn repeatedly (rather than sacrificing different groups of animals at various times, as is usually done with mice and rats). It was not technically possible to characterize the early distribution phase and the data are therefore treated in terms of a

single-compartment model. A plot of the elimination rate constant of BHC as a function of dose (Fig. 5) yields a curve which is similar to the liver:plasma distribution curves (Figs. 2 and 3). The smallest elimination rate constant occurred at a dose which yielded an initial BHC plasma concentration of 47 mcg./ml. on the average. This is in excellent agreement with the concentration at which the liver:plasma distribution ratio of BHC is at its miminum. These results are therefore consistent with previously obtained *in vitro* evidence (1) that the elimination rate constant of BHC is a function of its concentration at the site of biotransformation.

The question arises whether the *apparent* rate constant for BHC elimination changes continuously with time in parallel with the concentration-dependent changes in the distribution of the drug. The evidence so far is equivocal. The k_{app} values for BHC in rats, calculated from successive pairs of plasma concentration data obtained after administration of a single dose of BHC, are too scattered to justify any conclusion (Fig. 3). The experimental data in Fig. 4 and Table I suggest that $k_{app.}$ depends largely on initial distribution conditions. However, it has not been possible to demonstrate an actual crossover of BHC blood levels following a 5- and 60-mg./kg. dose, respectively. There seems to occur a change in the log concentration *versus* time slope about 20 hr. after administration of the large dose of BHC, but this could be due to several effects. It is possible that the rate of change in the distribution of BHC directly at the site of biotransformation (in microscopic terms) relative to the surrounding fluids and tissues lags behind the gross changes observed when drug concentrations are determined in the whole liver. A further complicating factor is the apparent self-inhibitory effect of BHC on its own metabolism (1). This and a previous study show that the self-inhibitory effect is small in the rat, though very pronounced in man and rhesus monkey where it accounts for a prolongation in the half-life of BHC with increasing dose (3). In the rat the dose-dependent distribution effect clearly predominates and large doses of BHC are eliminated relatively more rapidly than small doses.

There have been a number of clinical observations which are quite consistent with the principles apparent from this study. Aggeler and O'Reilly (9) have pointed out that the biologic half-lives of ethyl biscoumacetate, warfarin, and BHC in man are roughly proportional to their respective degrees of protein binding. Wiseman and Nelson (10) have shown a significant inverse correlation between the rate constants for the acetylation of various sulfonamides in man, and the degree of plasma protein binding of these drugs. Burns *et al.* have found in man that phenylbutazone, which is highly bound to plasma albumin, is eliminated much more rapidly following administration of very high doses (11). They suggested that this was due to the availability of a proportionately larger fraction of the drug in free form for biotransformation. Aggeler *et al.* (12) have found that administration of phenylbutazone with the coumarin anticoagulant warfarin decreased the half-life of the latter in man and potentiates its pharmacologic effect. This was thought to be due to the displacement of warfarin from its binding sites to plasma albumin, thus making more free drug available to drug-metabolizing enzymes and to specific sites of biologic action in the liver. Finally, a patient with hypoalbuminemia (1.6 g./100 ml. as compared to the normal value of about 4 g./100 ml.) was shown to be resistant to the anticoagulant effect of warfarin because the biologic half-life of warfarin in this patient was 6 to 7 hr. compared to the normal average half-life of about 2 days (13). These clinical observations, suggestive of the role of protein binding in the distribution and elimination of a drug, are directly confirmed by the results of this study.

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