polymeric material. Results of the experiments revealed that maximum sorption values occurred above the region of the critical micelle concentration for three of the four temperatures studied. Permeation experiments led to the evaluation of diffusion coefficients and permeability constants at several original concentrations of the cationic agent at four different temperatures. It was theorized that the binding of the benzalkonium chloride to the polyamide most likely occurred between the hydrophobic portions of the cation and the hydrocarbon regions of the nylon. The unusual sorption characteristics and the effect of original concentrations on the diffusion and permeation were attributed to the propensity of the benzalkonium chloride to form aggregates and micelles in aqueous mediums.

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Comparative Pharmacokinetics of Coumarin Anticoagulants V: Kinetics of Warfarin Elimination in the Rat, Dog, and Rhesus Monkey Compared to Man

RENPEI NAGASHIMA and GERHARD LEVY*

Abstract □ Small (1–4 mg./kg.) and large (10–12.5 mg./kg.) doses of sodium warfarin were administered intravenously to rats (Sprague-Dawley, male), dogs (mongrel, male), and monkeys (rhesus, male). Warfarin concentrations in the plasma declined exponentially with time in each species. The plasma half-life of warfarin was independent of dose in the dog, appears to decline slightly with increasing dose in the rat, and increases markedly with increasing dose in the monkey. Apparent volumes of distribution (dose/C_p^0) increased slightly with increasing dose in most of the animals. The half-life of warfarin, as observed in this study, increases in the order: rat < monkey < dog < man. It is shown that the dose-dependent elimination kinetics of bishydroxycoumarin, known to occur in man and monkeys, can be observed also with the other widely used coumarin anticoagulant, warfarin, when sufficiently high doses of the latter are administered. This effect is not seen clinically perhaps because the therapeutic dose range of warfarin is much lower than that of bishydroxycoumarin. Concomitant administration of a large dose (≥10 mg./kg.) of bishydroxycoumarin with sodium warfarin (2 mg./kg.) to monkeys tended to increase the half-life of warfarin. These observations are consistent with other indications suggesting that the two coumarin anticoagulants are subject to the same major biotransformation pathway(s).

Keyphrases □ Coumarin anticoagulants, elimination—pharmacokinetics □ Warfarin elimination—pharmacokinetics □ Pharmacokinetics comparison—warfarin elimination, rat, dog, monkey, man □ UV spectrophotometry—analysis □ Fluorometry—analysis

The pharmacokinetics of the coumarin anticoagulants have been the subject of considerable interest and study (reviewed in *Reference 1*). The kinetics of bishydroxycoumarin (BHC) elimination have been investigated in the rat, guinea pig, dog, and rhesus monkey (2), and a detailed multicompartmental analysis of BHC elimination has been attempted in man (3). More recent studies have resulted in the elucidation of the kinetics of the prothrombinopenic effect of the coumarin anticoagulants in man (4, 5). The present report is concerned with the pharmacokinetics of warfarin (3-α-phenyl-β-acetylethyl-4-hydroxycoumarin) elimination in the rat, dog, and rhesus monkey, as compared to man.

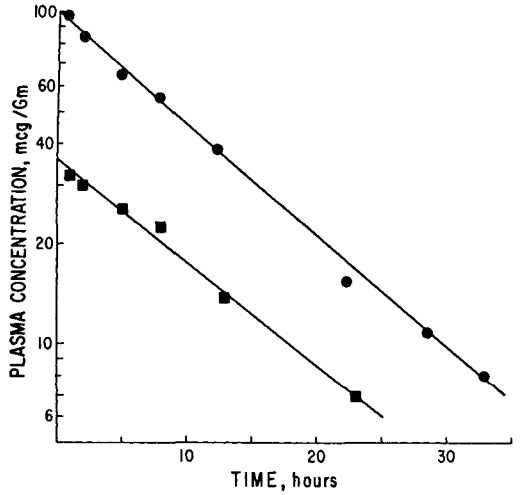

Figure 1—*Plasma concentration of sodium warfarin as a function of time in a rat (No. 7) which received a high (12.5 mg./kg.) and a low (4 mg./kg.) intravenous dose of the drug.*

EXPERIMENTAL

Materials—Warfarin solution for injection was prepared by dissolving microcrystalline sodium warfarin[1] in a sufficient volume of freshly distilled water to yield a 0.2–2% (w/v) solution. When BHC was injected concomitantly with sodium warfarin, a sufficient amount of the latter was dissolved in a solution containing 0.5% (w/v) bishydroxycoumarin.[2] The BHC solutions were prepared as described previously (2).

Animals—Rats, Sprague-Dawley, males, 350–450 g.; dogs, mongrel, males, 8–11 kg.; and monkeys, rhesus, males, 3–9 kg. were used. The animals had unrestricted access to food and water before and during the experiments.

Drug Administration and Collection of Plasma Samples—The coumarin drugs were administered intravenously in the morning. In certain experiments with dogs and monkeys, vitamin K_1,[3] was also administered intravenously, intramuscularly, or orally. Blood samples were collected at appropriate intervals and were processed to yield heparinized plasma samples as described previously (2). The plasma samples were stored at −15° until assayed.

Assay Methods—Warfarin concentration in the plasma was determined either by the UV spectrophotometric method of O'Reilly *et al.* (6) which has been shown to be specific for unchanged drug under the experimental conditions, or by a minor modification of the fluorometric method of Corn and Berberich (7). This modification involved the addition of one part by volume of 0.008 *M* phosphate buffer (pH 7.45) to ten parts of plasma sample. The buffer was necessary to obtain reproducible standard curves with aqueous solutions of warfarin. BHC plasma concentrations up to 60 mcg./ml. do not interfere with the fluorometric assay for warfarin. When plasma samples contained higher concentrations of BHC, they were diluted with human plasma (not water). Without such dilution, high concentrations of BHC cause some quenching of warfarin fluoresence. The recoveries of warfarin and blank values in terms of apparent sodium warfarin concentration (mcg./ml. plasma) from the plasma of the rat, dog, and monkey, as determined by the two assay methods, are listed in Table I. Possible metabolites of BHC and vitamin K_1 do not interfere with the assay for warfarin by the fluorometric method. The vitamin is known not to interfere in the spectrophotometric assay (8); this has been confirmed in the present study. The lack of interference by BHC or vitamin K_1 metabolites was evident from analyses of plasma samples from monkeys that had received either BHC or the vitamin, and to which known amounts of warfarin had been added.

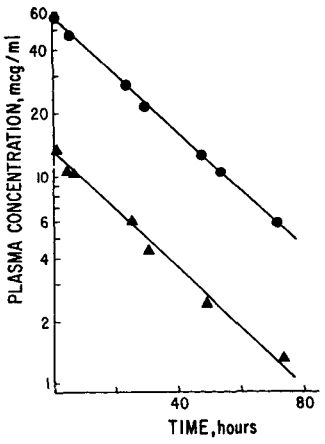

Figure 2—*Plasma concentration of sodium warfarin as a function of time in a mongrel dog (No. 7) which received a high (10 mg /kg.) and a low (2 mg./kg.) intravenous dose of the drug. Additional details in Table II.*

[1] Endo Laboratories Inc., Garden City, N. Y.
[2] Nutritional Biochemicals Co., Cleveland, Ohio.
[3] AquaMephyton or Mephyton, Merck Sharp and Dohme, West Point, Pa.

Table I—Comparison of Spectrophotometric and Fluorometric Methods for the Determination of Warfarin in Plasma

Plasma from	Spectrophotometry: % Recovery,[a] Mean ±*SD*	Spectrophotometry: Av. Blank,[b] mcg./ml.	Fluorometry: % Recovery,[a] Mean ±*SD*	Fluorometry: Av. Blank,[b] mcg./ml.
Rat	91 ± 3 (10)	5.3	—	—
Dog	—	—	96 ± 3 (13)	3.2
Monkey	93 ± 3 (9)	0.7	95 ± 3 (10)	<0.1

[a] Number of samples in parentheses. Warfarin concentrations in plasma, 2–40 mcg./ml. (rat) and 2–150 mcg./ml. (monkey) by spectrophotometry; 1.25–100 mcg./ml. by fluorometry. [b] In terms of apparent sodium warfarin.

The concentration of BHC in plasma was determined by a modification (1) of the spectrophotometric method of Axelrod *et al.* (9). A calibration chart was constructed to correct for the absorbance due to warfarin (which is about one-fifth that of an equal concentration of BHC). Vitamin K_1 and its possible metabolites do not interfere with the determination of BHC in plasma. All determinations were carried out at least in duplicate.

Determination of Plasma Half-Life—The plasma half-life ($t_{1/2}$) of warfarin was determined graphically from a plot of log plasma concentration *versus* time.

RESULTS AND DISCUSSION

Figure 1 shows the elimination of warfarin from the plasma of a rat after intravenous injection of a large and small dose of the drug. The plasma concentrations declined exponentially and there was only a slight (and possibly nonsignificant) increase in the apparent volume of distribution (V_d)[4] of warfarin with increasing dose. The half-life of warfarin appears to decrease slightly with increasing dose but the data are insufficient to establish a dose dependency with certainty. The elimination of large and small doses of warfarin in a dog is shown in Fig. 2. Plasma concentrations declined exponentially with a $t_{1/2}$ of approximately 21 hr. There was no pronounced change in the $t_{1/2}$ and V_d of warfarin with increasing dose in the dogs. The individual pharmacokinetic constants for warfarin elimination in the rats and dogs are listed in Table II.

The elimination of warfarin by the monkeys was dose-dependent (Figs. 3 and 4, and Table III) in that the $t_{1/2}$ increased with increasing dose although the plasma concentration declined exponentially (disregarding minor fluctuations) at all dose levels. Marked apparent increases in V_d with increasing dose are evident in Monkeys 1, 2, and 3 (Table III). However, this apparent dose-dependence in V_d may be an artifact and could be the result of an initial decrease with time in the elimination rate constant of warfarin following administration of the higher doses. Thus, it is possible that the initial curvature of the warfarin concentration *versus* time plot observed in Monkey 1 (Fig. 3) with the highest dose (10 mg./kg.) reflects not

[4] V_d = dose/C_p^0 where C_p^0 is the apparent initial concentration of sodium warfarin in the plasma which is determined by extrapolating the terminal linear portion of a log concentration *versus* time plot to zero time.

Table II—Pharmacokinetic Constants for Warfarin Elimination from Plasma in Rats and Dogs

Animal No.	Body wt., kg.	Date Dosed	Dose,[a] mg./kg.	$t_{1/2}$, hr.	Dose/ C_p^{0}[b] ml./kg.	Assay[c] Method
			Rat			
6	0.39	6/14	12.5	9.8	130	S
	0.43	6/21	4.0	11.0	115	S
7	0.37	6/14	4.0	9.7	110	S
	0.36	6/21	12.5	9.0	120	S
			Dog			
6	10.0	11/21	10[d]	24	140	F
	10.3	12/12	2[d]	22	140	F
7	8.4	11/21	2[d]	21	155	F
	8.1	12/12	10[d]	22	175	F

[a] Dose as sodium salt. The drug was administered intravenously. [b] C_p^0 is the apparent initial concentration of sodium warfarin in plasma estimated by back-extrapolation of the terminal linear portion of a log plasma concentration *versus* time plot. [c] S, spectrophotometry; F, fluorometry. [d] Doses of vitamin K_1 (10 mg.) were administered orally on Days −1, 0, and 2 following warfarin.

(only) the so-called distribution phase but (also) a relatively more rapid initial elimination phase. The degree of reproducibility of warfarin-elimination kinetics is shown in Monkeys 4 and 5 following administration of 2 mg./kg. on two different occasions (Table III). Another point of interest in the data obtained with monkeys is the *suggestion* of diurnal variation following administration of the higher doses (Figs. 3 and 4). As described in the subsequent paragraph, this cannot be attributed to assay problems. There was no indication of an effect of vitamin K_1 on warfarin elimination; this is consistent with similar observations in man (8). The pharmacokinetic data obtained by spectrophotometric assay are in reasonable agreement with those obtained by the fluorometric assay method (Table III), despite recent evidence that one of the four hydroxylated metabolites of warfarin may possibly interfere with the fluorometric assay for warfarin (22).

The results of the study on the effect of concomitantly administered BHC on warfarin elimination in the monkeys are shown in

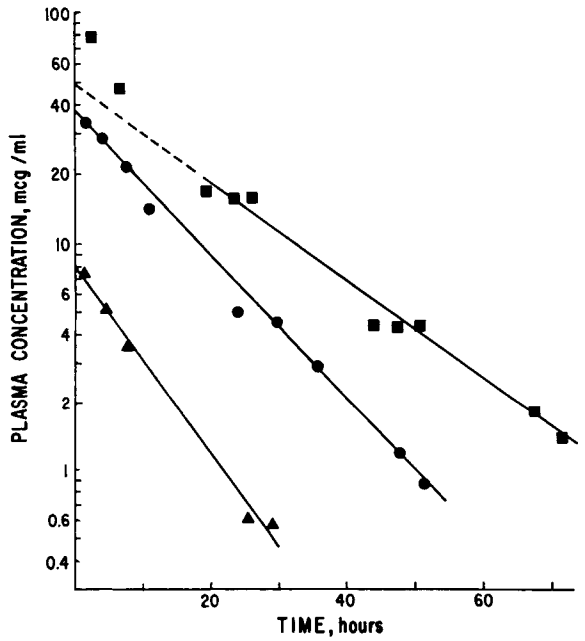

Figure 3—*Plasma concentration of sodium warfarin as a function of time in a rhesus monkey (No. 1) which received a high, intermediate, and low intravenous dose of the drug. The dates isted in this and the other figure legends refer to the time of warfarin sodium administration. Key: ▲, 12/28/66, 1 mg./kg.; ●, 1/4/67, 4 mg./kg.; ■, 1/17/67, 10 mg./kg. Additional details in Table III.*

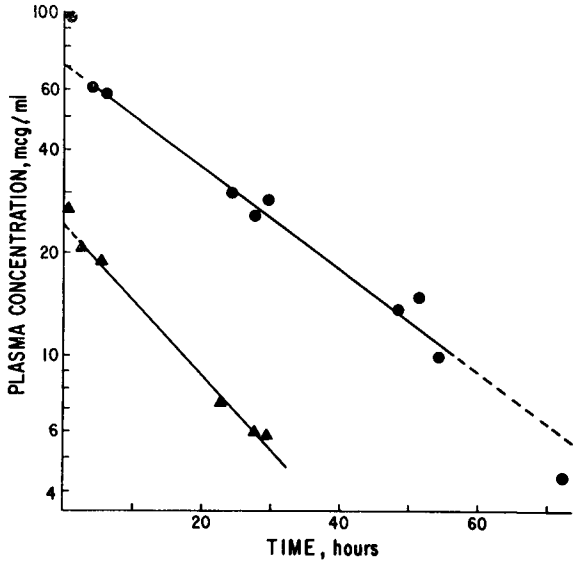

Figure 4—*Plasma concentration of sodium warfarin as a function of time in a rhesus monkey (No. 3) which received a high (10 mg./kg.) and a low (2 mg./kg.) intravenous dose of the drug. Additional details in Table III.*

Fig. 5 and Table IV. The two animals (Nos. 4 and 5) received the drugs in opposite sequence at an interval of 2 weeks (except for the first interval with Monkey 4 which was 9 weeks). It appears that BHC inhibited somewhat the elimination of warfarin. It is noteworthy that, in spite of some fluctuations, the overall plasma concentration decline was exponential even when BHC was coadministered (Fig. 5). The apparent inhibitory effect of BHC on warfarin elimination, coupled with the previously mentioned dose-dependence of the $t_{1/2}$ of warfarin similar to that found for BHC in man and monkeys (2, 10, 11), suggests that warfarin and BHC are subject to the same type of elimination process(es).

When BHC was coadministered with warfarin, the plasma concentrations of BHC were also determined. In Fig. 6, the concentrations of both BHC and warfarin in the plasma are shown as functions of time. These data were obtained on two different occasions in each of the two monkeys (Nos. 4 and 5). The encircled data points in Fig. 6 show the coincidence of peculiar fluctuations in the plasma concentration curves of both drugs. It should be pointed

Table III—Pharmacokinetic Constants for Warfarin Elimination from Plasma in Monkeys[a]

Animal No.	Body wt., kg.	Date Dosed	Dose,[b] mg./kg.	$t_{1/2}$, hr.	Dose/ C_p^{0}[c] ml./kg.	Assay[d] Method
1	5.0	12/28	1	7.2	125	S
	5.0	1/4	4	9.5	105	S
	5.2	1/17	10[e]	14.0	205	S
2	6.4	2/7	10[e]	9.2	215	S
	8.6	4/25	1[e]	7.9	160	S
3	6.1	11/28	10[f]	20.0	140	F
	6.1	12/12	2[f]	13.6	84	F
4	5.7	11/28	2[f]	10.5	98	F
	5.7	12/12	10[f]	14.5	135	F
	5.9	2/13[g]	2[f]	9.6	120	F
5	3.6	1/30[g]	2[f]	10.5	160	F
	3.4	2/27[h]	2[f]	10.5	130	F

[a] Monkeys 1 and 2 are the same as used in a previous study on BHC elimination (2). [b] Dose as sodium salt. The drug was administered intravenously. [c] For explanation of C_p^0, see *Footnote b* under Table II. [d] S, spectrophotometry; F, fluorometry. [e] Vitamin K_1 (0.5 mg./kg.) was administered concomitantly intravenously. [f] Vitamin K_1 (2 mg./kg.) was administered concomitantly intravenously. [g] This experiment was preceded 2 weeks earlier by another in which the following drugs were administered: 2 mg. sodium warfarin/kg. i.v., 10 mg. BHC/kg. i.v., and 2 mg. vitamin K_1/kg. i. m. [h] This experiment was preceded 2 weeks earlier by another in which the following drugs were administered: 10 mg. BHC/kg. i.v. and 2 mg. vitamin K_1/kg. i.m. on Day −1, and 2 mg. warfarin/kg. i.v., 10 mg. BHC/kg., i.v., and 2 mg. vitamin K_1/kg. i.m. on Day 0.

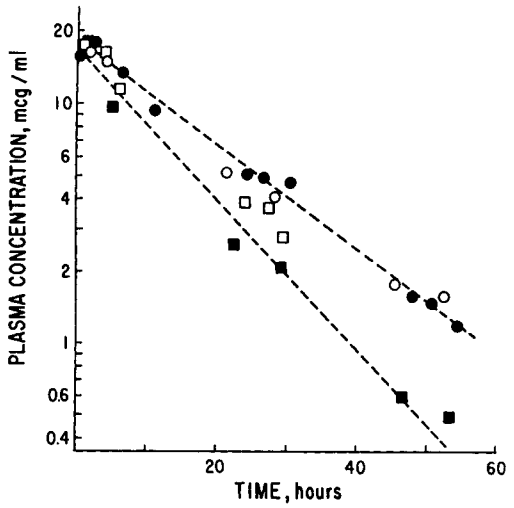

Figure 5—*Plasma concentration of sodium warfarin as a function of time in a rhesus monkey (No. 4) which received four intravenous doses (2 mg./kg.) of sodium warfarin with and without intravenous coadministration of BHC. Key: □, 11/28/67, control without BHC; ●, 1/30/68, with 10 mg. BHC/kg.; ■, 2/13/68, control without BHC; ○, 2/27/68, with 10 mg. BHC/kg. 24 hr. before and 5.4 mg. BHC/kg. at the time of sodium warfarin administration. Additional details in Table IV.*

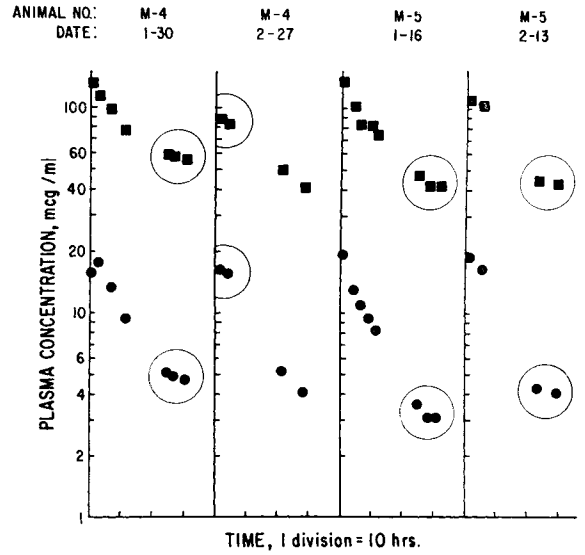

Figure 6—*Plasma concentrations of concomitantly administered BHC (■) and sodium warfarin (●) as functions of time in two rhesus monkeys (No. 4 and No. 5) on two different occasions. Additional details in Table IV. The circles emphasize the similarities in the plasma concentration patterns of the two drugs.*

out that the assay methods for the two drugs are completely different; spectrophotometry for BHC and fluorometry for warfarin. While the occurrence of such fluctuations in the plasma concentration curves of any one drug might be ascribed to assay variation, the consistent parallelism in the warfarin and BHC data rules out such a possibility. They may be due to certain changes in body distribution of the drugs (perhaps mediated by changes in plasma volume, or in protein binding due to variations in the fatty acid concentrations in the plasma), or they may reflect variations in the kinetics of biotransformation. Regardless of the cause of these effects, it is significant that there seems to be a factor which simultaneously affects the plasma concentrations of both drugs.

Comparative Aspects of the Pharmacokinetics of Warfarin and BHC—The results of the present study of the kinetics of warfarin elimination in rats, dogs, and rhesus monkeys, and the results obtained by others in man (8, 12), are summarized in Table V. In addition, there are listed the results of previous studies of BHC elimination in several animal species (2, 13) and in man (10, 11, 14, 15). The table can therefore serve as a basis for comparing the pharmacokinetics of the two major coumarin anticoagulants in different animal species. Such a comparison must of course take into account the limited number of animals studied, the possibility of strain and sex differences, differences in experimental conditions, and the fact that the data do not permit a multicompartment analysis. Several reports which appeared after the conclusion of the present study provide information which is pertinent to these considerations. Pyörälä and Nevanlinna found an average warfarin half-life of 6 hr. in highly inbred strains of Sprague-Dawley and Wistar rats (16). These workers were also able to develop, by selective inbreeding, two strains of Sprague-Dawley rats showing an

Table IV—Effect of Concomitant Administration of Bishydroxycoumarin (BHC) on Warfarin Elimination from Plasma in Monkey[a]

Animal No.	Body wt., kg.	Co-administration of BHC	Date Dosed	$t_{1/2}$, hr.	Dose/C_p^0, ml./kg.
4	5.7	no	11/28	10.5	98
	5.9	yes[b]	1/30	13.7	110
	5.9	no	2/13	9.6	120
	6.1	yes[c]	2/27	13.7	110
5	4.0	yes[b]	1/16	12.1	125
	3.6	no	1/30	10.5	160
	3.6	yes[c]	2/13	11.0	90
	3.4	no	2/27	10.5	130

[a] Sodium warfarin dose: 2 mg./kg. i.v. Plasma warfarin concentrations were determined by fluorometry. [b] Coadministration of BHC 10 mg./kg. i.v. and vitamin K_1 2 mg./kg. i.m. [c] Coadministration of BHC 10 mg./kg. i.v. and vitamin K_1 2 mg./kg. i.m. 24 hr. before, and BHC ~6 mg./kg. i.v., and vitamin K_1 2 mg./kg. i.m. at the time of warfarin administration.

Table V—Comparison of Average Pharmacokinetic Constants for Warfarin and Bishydroxycoumarin Elimination from Plasma in Different Animal Species

Warfarin			Bishydroxycoumarin		
Dose,[a] mg./kg.	$t_{1/2}$, hr.	V_d,[b] ml./kg.	Dose,[a] mg./kg.	$t_{1/2}$, hr.	V_d,[b] ml./kg.
			Rat		
4 (2)	10.4	113	2(2)[c]	5.6	89
12.5(2)	9.4	125	5(3)[d]	6.4	107
			20(2)[c]	4.8	98
			~60(3)[d]	3.7	187
			Dog		
2 (2)	22	148	1(3)[c]	58	119
10 (2)	23	158	8(3)[c]	64	121
			Monkey		
1 (2)	7.6	143	1(2)[c]	5.9	77
2 (3)	11.3	113			
10 (4)	14.4	174	10(2)[c]	9.0	81
			Man		
1.5(2)[e]	29	122	2.1(1)[f]	10	200
3.0(2)[e]	37	129	8.6(1)[f]	32	130
1.5(30)[g]	43 ± 10	—	2.0(56)[h]	23.6 ± 5.8	—
			100 mg. total dose (5)[i]	22 ± 6	—
			150 mg. total dose (5)[i]	32 ± 7	—
			5.0 (8)[i]	40(18–190)	~140

[a] Number of animals or subjects in parentheses. [b] Apparent volume of distribution = dose/C_p^0. For explanation of C_p^0, see *Footnote b* under Table II. [c] From a previous study (2). [d] From a previous study (13). [e] Data on the same two subjects from O'Reilly *et al.* (8) after intravenous administration. [f] From O'Reilly *et al.* (11), after intravenous administration. [g] From O'Reilly and Aggeler (12), after oral administration. [h] From Motulsky (14), after oral administration. [i] From Schrogie and Solomon (15), after oral administration. [j] From Weiner *et al.* (10), after intravenous administration.

average warfarin half-life of 5 and 30 hr., respectively. Pyörälä found a sex difference in the half-life of warfarin in random-bred Sprague-Dawley rats, with females showing a somewhat longer half-life than males (17). Ikeda *et al.* (18) reported data on plasma warfarin concentrations in young (50 g.) Sprague-Dawley rats which yield a half-life of about 9 hr. Hunninghake and Azarnoff (19) studied warfarin elimination in male and female mongrel dogs and observed an average half-life of about 29 hr. The results of Ikeda *et al.* (18) and those of Hunninghake and Azarnoff (19) agree well with the results of the present study. This is particularly significant because these workers used assay methods different from those used in this investigation. It should be noted that only in the present study was warfarin administered intravenously. This is of some significance with a poorly water-soluble drug such as warfarin.

A review of the data summarized in Table V reveals certain similarities in the pharmacokinetics of warfarin and BHC. The rat and the monkey eliminate both drugs more rapidly than does the dog or man. The monkey shows a definite dose-dependence in the elimination of BHC and warfarin, with larger doses being eliminated more slowly. The same type of dose-dependence has been found in the elimination of BHC in man (11). However, no such dose-dependence has been observed in the elimination of warfarin in man. It seems that this may be due to the greater potency of warfarin, and the consequent use of much lower doses of this drug. In fact, re-evaluation of data on large doses (8, 20)[5] suggests that the half-life of warfarin in man also increases with increasing dose (data on the same two subjects in Table V). This dose-dependence was demonstrated readily in monkeys given vitamin K_1, since the vitamin protects the animals from warfarin-induced hemorrhages and therefore makes possible the administration of very high doses of the anticoagulant.

It appears that the rhesus monkey is the most suitable (of the species studied) for further investigations of the pharmacokinetics of the coumarin anticoagulants since this animal and man are quite sensitive to the apparent inhibitory effect of the coumarin anticoagulants on their own biotransformation (3). The rat, on the other hand, reflects primarily the effects of concentration-dependent changes in the distribution of a coumarin drug on the biotransformation kinetics (21) and is relatively resistant to the apparent self-inhibitory effect in the biotransformation of this class of drugs (13).

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