In Vivo and *In Vitro* Availability of Commercial Warfarin Tablets

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Abstract □ Rate of absorption and bioavailability of warfarin following oral administration of four different commercial warfarin tablets was assessed by measurement of plasma concentrations of warfarin followed by pharmacokinetic and statistical analysis of the resulting data. In Study No. 1, results obtained with five 5-mg. tablets were compared with those obtained with one 25-mg. tablet, all tablets being made by the same manufacturer. The rate of absorption was about twice as fast from the five 5-mg. tablets as from the one 25-mg. tablet. Relative absorption from the one 25-mg. tablet was only about 80%, compared with 100% from the five 5-mg. tablets. In Study No. 2, three 5-mg. tablets, prepared by three different manufacturers, were compared in a crossover study in 12 normal subjects at a two-tablet (10-mg.) dose. The rate constant for absorption varied over about a fourfold range, but all three tablets were equally available and the entire dose of warfarin was absorbed, apparently, after each treatment. Results obtained in man correlated excellently with results of *in vitro* rate of dissolution tests but not with disintegration times of the tablets. The results will aid in delineating the critical area for *in vitro* dissolution rates of warfarin tablets prepared similarly to the commercial products and in quality control procedures.

Keyphrases □ Warfarin tablets—*in vitro*, *in vivo* availability □ Bioavailability—warfarin tablets □ Absorption-rate constants—warfarin tablets □ Pharmacokinetics—warfarin absorption □ Tablets, warfarin—plasma concentration comparison □ Dissolution, absorption-rate correlation—warfarin tablets

The importance of performing both clinical studies and *in vitro* rate of dissolution tests on different manufacturers' formulations of the same drug was recently stated in a "White Paper on the Therapeutic Equivalence of Chemically Equivalent Drugs," prepared by a subcommittee of the Policy Advisory Committee, Drug Efficacy Study of the National Research Board, National Academy of Sciences–National Research Council (1). Minimum variability of absorption rates in man and complete physiological availability are desired characteristics for all drugs, but these properties are especially important for an anticoagulant such as warfarin.

Previous investigations were made of the *in vitro* dissolution and *in vivo* absorption characteristics of different oral dosage forms of both warfarin (acid) and its sodium salt (2, 3). These studies strongly suggested that absorption of warfarin in man is dissolution-rate controlled. However, both these studies were performed using 100-mg. doses of warfarin, and the commercial formulations used were not identified. It appeared desirable to compare plasma concentrations of warfarin in man following oral administration of several commercial tablets at dose levels in the normal therapeutic range. Warfarin presents a particularly interesting example in this area since plasma concentrations of warfarin have already been indirectly correlated with efficacy as measured by depression of "prothrombin complex activity" using a pharmacokinetic approach (4).

This report compares plasma concentrations of warfarin (acid) measured in two different crossover studies. In the first study, six subjects ingested one 25-mg. tablet or five 5-mg. tablets of sodium warfarin; both tablets were made by the same manufacturer. Plasma samples were analyzed independently by two different methods of analysis. In the second study, 12 subjects ingested 10-mg. label doses of sodium or potassium warfarin administered as two tablets of three different manufacturers' kinds of warfarin. *In vitro* rate of dissolution tests were performed on five individual tablets of each lot of tablets tested clinically. Three lots of tablets containing 5 mg. of sodium warfarin and three lots of tablets containing 25 mg. of sodium warfarin were prepared; some of these tablets were shown to release the warfarin *in vitro* much faster than any commercial tablet studied.

EXPERIMENTAL

Screening of Subjects—Subjects screened to become members of each of the two panels received a drug history and physical examination which included measurement of blood pressure, pulse, and respiration. Hematology measurements included hemoglobin, hematocrit, mean corpuscular hemoglobin concentration, white cell count, and differential. Blood chemistry measurements included serum creatinine, bilirubin (both free and total), serum glutamic oxalacetic transaminase, and alkaline phosphatase. Each prospective panel member also had a urinalysis, chest X-ray, and electrocardiogram. In the second study, prothrombin time was measured during the screening a few days before the study began and 96 hr. after the last dose of warfarin was administered.

In the first study, 10 subjects were screened and seven were utilized. In the second study, it was necessary to screen 20 subjects to obtain a satisfactory panel of 12 "normal" subjects. Only those subjects who had all parameters in the normal range were selected.

Subjects Selected—*Study No. 1*—Subject 6, who received Treatment B (one 25-mg.) in Phase I was replaced by Subject 6*a* who received Treatment A (five 5-mg.) in Phase II. The other five subjects received both treatments as scheduled. Subjects 1 and 4 were females, and the remainder were males. Group I consisted of Subjects 1, 2, and 3 in both Phases I and II; Group II consisted of Subjects 4, 5, and 6 in Phase I and Subjects 4, 5, and 6*a* in Phase II. The group number, average age and range of ages, and average body weight and range of body weights were as follows: I, 25 (21–30) years, 68 (54.5–90.5) kg.; II (Phase I), 27 (23–31) years, 70.5 (59.0–77.5) kg.; and II (Phase II), 28 (23–31) years, 71.5 (59.0–77.5) kg.

Study No. 2—Group I consisted of Subjects 1 through 4, Group II consisted of Subjects 5 through 8, and Group III consisted of Subjects 9 through 12. Subjects 2 and 12 were females, and the

Table I—Dosage Schedules Employed in the Two Clinical Studies

Group	Subjects	Study No. 1 Phase I	Study No. 1 Phase II
I	1, 2, 3	A (five 5-mg.)	B (one 25-mg.)
II	4, 5, 6	B (one 25-mg.)	
	4, 5, 6[a]		A (five 5-mg.)

Group	Subjects	Study No. 2 Phase I	Study No. 2 Phase II	Study No. 2 Phase III
I	1 to 4	A (two 5-mg.)	C (two 5-mg.)	D (two 5-mg.)
II	5 to 8	C (two 5-mg.)	D (two 5-mg.)	A (two 5-mg.)
III	9 to 12	D (two 5-mg.)	A (two 5-mg.)	C (two 5-mg.)

remainder were males. The statistics (as already explained) were as follows: I, 24 (23–32) years, 72.0 (59.0–90.0) kg.; II, 24 (21–31) years, 78.0 (68.0–92.0) kg.; and III, 24 (21–27) years, 74.5 (60.5–94.0) kg. Four subjects were common to both studies; their subject numbers in Study No. 2 followed by their subject numbers in Study No. 1 in brackets were as follows: 2 (4), 3 (3), 6 (5), and 12 (1).

Informed consent forms were signed by all subjects who participated in these studies.

Protocols—All subjects received no barbiturates or other known enzyme-inducing agents for a period of 30 days and no medication other than the prescribed medication for study for a period of 7 days preceding initiation of the studies and during the studies. Subjects were fasted overnight and for 4 hr. postadministration of warfarin. On the mornings medication was administered, each subject drank 240 ml. (8 oz.) of water within the 1st hr. after arising and 240 ml. of water when the tablets were ingested at zero time for each subject (between 8 and 9 a.m., accurately determined in each case). Tablets were swallowed whole.

During Study No. 1, each subject was administered two tablets of menadiol diphosphate (tetrasodium salt)[1], 5 mg., the day before administration of warfarin, 4 hr. following the dose of warfarin, and 3 days following the dose of warfarin. In Study No. 2, no medication other than warfarin was administered. All subjects were ambulatory during the daytime and performed their normal jobs in the hospital.

In Study No. 1, 20 ml. of whole blood, and in Study No. 2, 15 ml. of whole blood, was taken from a forearm vein at zero time and at 1, 4, 8, 12, 24, 48, 72, and 96 hr. postdosing. The blood was drawn into one or two vacutainer tubes containing ethylenediaminetetraacetic acid as anticoagulant. Each blood sample was centrifuged as rapidly as possible after collection, and the plasma was quick-frozen and kept in the frozen state (about −20°) until just prior to assay. All samples were double labeled with paste labels and tags bearing subject numbers, phase, collection time in hours, and date. In both studies, treatments were separated by a period of 3 weeks (504 hr.) which is equivalent to about 10 or more average half-lives of warfarin in plasma.

Treatments and Schedules—Four tablets, designated A, B, C, and D, were studied[2]. In Study No. 1, treatments were A (five 5-mg.), meaning five 5-mg. tablets of type A, and B (one 25-mg.), meaning one 25-mg. tablet of type B, both of which were made by the same manufacturer. In Study No. 2, treatments were A (two 5-mg.), meaning two tablets of type A; C (two 5-mg.), meaning two tablets of type C; and D (two 5-mg.), meaning two tablets of type D. Dosage schedules are given in Table I.

Assay of Plasma Samples—In Study No. 1, 4-ml. aliquots of each plasma sample were independently analyzed by a modification of the simple extraction–UV spectrophotometric method of O'Reilly *et al.* (5) (Method E) and by a TLC method similar to that of Lewis and Ilnicki (6) but employing a UV spectrophotometric rather than a fluorescence end-point (Method T). The details of these methods were described in a previous publication (7). Menadiol diphosphate (tetrasodium salt) was shown not to interfere in either method.

In Study No. 2, a second modification of the simple extraction–UV spectrophotometric method of O'Reilly *et al.* (5) was employed. In this method, 40 ml. of 1,2-ethylene dichloride, 7 ml. of plasma, 3 ml. of distilled water, and 2 ml. of 2.6 *N* hydrochloric acid were shaken for 10 min. in a Kahn shaker in a 100-ml. bottle fitted with a polyethylene-lined screw cap. The 1,2-ethylene dichloride layer was separated and washed with 10 ml. of 0.1 *M* phosphate buffer, pH 7.2. A 30-ml. aliquot of the washed 1,2-ethylene dichloride extract was extracted with 5 ml. of 2.5 *N* sodium hydroxide solution.

The absorbances of the alkaline aqueous extract at 308 and 360 nm. were read in a 7.5-cm. pathlength cell in a Gilford single-beam UV spectrophotometer. The instrument was zeroed for each extract at each wavelength by adjusting the slit width with water in the same cell as used to measure the absorbance of the sample extracts. The plasma concentrations were calculated from the formula:

$$\text{plasma concentration of warfarin (acid) in mcg./ml.} = \frac{(As_{308} - As_{360}) - (Ab_{308} - Ab_{360})}{0.320} \quad \text{(Eq. 1)}$$

where *As* is the absorbance of the extract derived from plasma obtained after dosing with warfarin, and *Ab* is the absorbance of the extract derived from the same subject's zero hour (predose) plasma.

As a quality control procedure, a spiked blood bank plasma sample (spiked at 2 mcg. warfarin/ml.) and a blank blood bank plasma sample were carried through the assay procedure with each daily batch. A daily batch constituted all the plasma samples of one particular subject on one phase of the crossover.

The factor 0.320 in Eq. 1 is based on extensive assay standardization, described in the previous report (7), employing reference standard warfarin (acid) USP.

Assay of Tablets—Each lot of tablets was assayed by the official USP XVIII method, using a group of 20 tablets from each bottle used in the clinical studies. Instead of assaying only one aliquot of powder derived from the 20 tablets, as prescribed by the USP, three different aliquots of powder were taken to obtain an indication of assay error. In addition, five individual tablets from each lot were assayed by a modification of the USP XVIII method. In the modified method, the single tablet was dissolved in 0.1 *N* sodium hydroxide by agitation; then the solution was shaken for 15 min. The volume of sodium hydroxide solution to be acidified and back-extracted into chloroform was adjusted to 15 ml. for the 5-mg. tablets and 3 ml. for the 25-mg. tablet. Appropriate calculations made from the observed absorbances enabled the total warfarin in individual tablets to be assigned.

Solubility of Warfarin (Acid) in 0.1 *N* Hydrochloric Acid—Six determinations of the solubility of warfarin in 0.1 *N* hydrochloric acid were made at both room temperature (about 25°) and at 37°. For each temperature, two bottles contained 5 mg., two bottles contained 25 mg., and two bottles contained 50 mg. of sodium warfarin. One hundred milliliters of 0.1 *N* hydrochloric acid was added to each bottle, and the bottles were equilibrated at the appropriate temperatures in a shaking bath overnight. Similar net absorbances ($A_{308} - A_{360}$) were obtained from each bottle at a given temperature.

***In Vitro* Rate of Dissolution Studies**—The pH of the fluids and exposure times were the same as those used by O'Reilly *et al.* (2). Single tablets were tested individually from each lot. Each tablet was exposed initially to 900 ml. of 0.1 *N* hydrochloric acid for 30 min. After 30 min, 100 ml. of a strong phosphate buffer was added to raise instantaneously to and maintain the pH at 7.5 ± 0.1 for the rest of the dissolution test. The dissolution assembly was similar to that described by Poole (8), being a three-necked, round-bottom, 1-l. flask. The stirring shaft was 25.4 cm. (10 in.) long; the stirring paddle was half-moon shaped, being 3.08 cm. (2 in.) wide and 1.91 cm. (0.75 in.) deep at the widest part of the arc. Both shaft and paddle were made of stainless steel. The shaft and paddle were centered in the middle hole of the flask and positioned so that the bottom of the paddle was exactly 2.5 cm. from the bottom of the flask. The shaft was rotated at 50 r.p.m. by means of a GT21 laboratory mixer and motor controller[3].

[1] Synkayvite, Roche.

[2] Tablet A was Coumadin, sodium, 5 mg. (Endo Laboratories, Inc.), Lot No. BA500A; Tablet B was Coumadin, sodium, 25 mg. (Endo Laboratories, Inc.), Lot No. 7H0212; Tablet C was Athrombin-K, 5 mg. (Purdue-Frederick), Lot No. K687355; and Tablet D was Panwarfarin (sodium), 5 mg. (Abbott Laboratories), Lot No. 830-0755-21.

[3] Gerald K. Keller Co.

Table II—Plasma Warfarin Concentrations[a] (Micrograms/Milliliter) Measured in Study No. 1 following 25-mg. Doses of Warfarin Sodium

Subject	Treatment	Analytical Method	Body Weight, kg.	Hours 1	4	8	12	24	48	72	96
1	A (five 5-mg.)	E[b]	59.0	2.84	4.08	3.19	3.25	2.69	1.47	0.81	0.48
		T[c]		2.85	4.54	2.80	3.22	2.25	1.67	0.93	0.62
	B (one 25-mg.)	E		2.93	3.86	3.17	3.03	2.31	1.60	1.04	0.53
		T		2.96	3.97	3.24	3.20	2.10	1.60	0.93	0.62
2	A (five 5-mg.)	E	54.4	2.19	3.36	3.21	2.66	1.90	1.16	0.61	0.34
		T		2.35	2.71	2.66	2.69	2.44	1.26	0.63	0.59
	B (one 25-mg.)	E		0.14	0.86	2.34	2.29	1.80	1.01	0.60	0.33
		T		0.14	0.76	2.23	2.32	1.77	0.96	0.52	0.34
3	A (five 5-mg.)	E	90.3	2.44	2.43	1.89	1.86	1.70	1.22	0.84	0.70
		T		2.71	2.54	2.00	1.85	1.47	0.96	0.58	0.34
	B (one 25-mg.)	E		1.70	2.01	1.86	1.83	1.49	0.97	0.72	0.53
		T		1.35	1.89	1.68	1.61	1.35	0.92[d]	0.67	0.41
4	A (five 5-mg.)	E	59.0	1.38	3.73	2.91	2.96	2.15	1.28	0.83	0.48
		T		1.63	3.42	2.95	3.11	2.11	1.24	0.61	0.53
	B (one 25-mg.)	E		0.48	1.89	2.22	2.44	1.92	1.02	0.54	0.16
		T		0.70	2.21	2.40	2.78	1.98	1.36	0.83	0.44
5	A (five 5-mg.)	E	77.1	2.57	2.54	2.62	2.18	1.37	0.99	0.58	0.34[e]
		T		2.86	2.64	2.62	2.26	1.57	1.13	0.56	0.51
	B (one 25-mg.)	E		0.79	1.35	1.57	1.86	1.42	1.22	0.91	0.68
		T		0.46	1.20	1.58	1.77	1.25	1.05	0.70	0.47
6*a*	A (five 5-mg.)	E	77.1	2.62[f]	2.89	2.42	2.30	1.93	1.74	1.02	0.82
		T		2.50	3.02	2.38	2.44	1.81	1.10	0.95	0.62
6	B (one 25-mg.)	E	74.8	2.01	2.30	2.08	2.10	1.39	0.83	0.53	0.42
		T		1.89	2.32	1.76	1.76	1.08	0.86	0.49	0.32

[a] All zero-hour plasmas were zero. Plasma hemolyzed; could not be assayed. [b] E = modified O'Reilly extraction method. [c] T = TLC method. [d] Value estimated from semilogarithmic plot of 24-, 72-, and 96-hr. plasma concentrations. (Plasma hemolyzed; could not be assayed.) [e] Value estimated from semilogarithmic plot of 48- and 72-hr. plasma concentrations. (Plasma hemolyzed; could not be assayed.) [f] Value estimated from 0-, 4-, 8-, and 12-hr. plasma concentrations by nonlinear interpolation.

The dissolution fluids were maintained at 37 ± 1° by means of a constant-temperature bath. Fluid was continuously circulated through a small syringe barrel fitted with glass wool and small glass beads (one end of the barrel being immersed in the dissolution medium) into 0.29 cm. (0.125 in.) i.d. silicone tubing, through a Sigmamotor pump, then through a silica flowcell in a Gilford UV spectrophotometer, and back into the three-necked dissolution flask again. A V-shaped glass tube filled partly with glass beads was placed in the silicone tubing line to act as a debubbling device. For the 5-mg. tablets, a 10-mm. lightpath was used; for the 25-mg. tablets, a 1-mm. pathlength flowcell was used. The rate of flow of fluid in the system was of the order of 70 ml./min.

The absorbance of the filtered dissolution medium was read manually at 308 nm., the wavelength of maximum absorbance of warfarin, at very frequent intervals. When greater than 85% of the warfarin had dissolved, the stirring speed was increased to 277 r.p.m. until a constant absorbance value was obtained. Reference standard warfarin (acid) USP was used for calibration purposes. Values obtained with this standard were then used to calculate the equivalent amount of sodium or potassium warfarin dissolved at different times from the tablets. The amount of sodium or potassium warfarin corresponding to the constant absorbance value was used as the denominator to calculate the percent of warfarin sodium or potassium dissolved at each sampling time.

Disintegration Times—Disintegration times were determined using at least 12 tablets of each lot. The USP XVII disintegration apparatus, without plastic disks, and water at 37° were employed.

Interpretation of Results—Symbolism employed is as follows:

C = plasma concentration of warfarin (acid) at time t after administration (micrograms/milliliter).

D = dose of warfarin (acid) based on the official assay of 20 tablets (milligrams).

F = fraction of the dose of warfarin that was absorbed (dimensionless).

Peak = observed peak plasma concentration of warfarin (micrograms/milliliter).

W = body weight of an individual subject (kilograms).

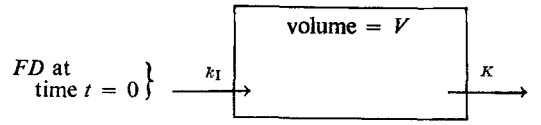
A = estimated area under the plasma concentration curve from time zero to infinite time. This is sometimes symbolized by area $0 \rightarrow \infty$ and was estimated by the usual method (4). The dimensions are (micrograms/milliliter) × hours.

$T_{1/2}$ = half-life calculated from the terminal plasma concentrations by the method of least squares when ln C was plotted *versus* t. Only those points that appeared to be randomly distributed about a straight line were utilized. Usually the earliest time value used was 24 or 48 hr. The line fitting the points has the equation: $\ln C = \ln C^0 - (0.693/T_{1/2})t$. $T_{1/2}$ is expressed in hours. In Table VIII, it is shown that $0.693/\beta$, where β was obtained by two-compartment analysis, is essentially the same as $T_{1/2}$ estimated above; this is not necessarily true generally but is true with these warfarin data.

Normalized area = $0.693A/[(T_{1/2})(D/W)]$, which is the same as the equation of Wagner (9).

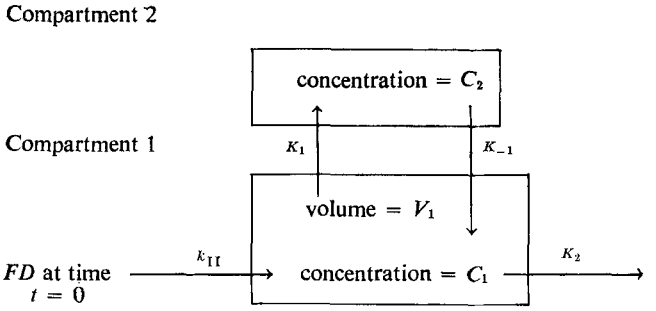
V_d = apparent volume of distribution of warfarin when the two-compartment open model is viewed as a one-compartment open model. This has formerly (10) been termed $V_{d\ \text{area}}$. It may be shown that $V_d = V_1\alpha/K_{-1}$, where V_1, α, and K_{-1} are defined below. V_d is expressed in liters. V_d/W is the V_d expressed as a fraction of body weight.

The one-compartment open model with first-order absorption may be written as shown in Scheme I:



Scheme I

The two-compartment open model with first-order absorption may be written as shown in Scheme II:



Scheme II

where V = apparent volume of distribution obtained by one-compartment analysis (Scheme I); K = apparent elimination-rate constant obtained by one-compartment analysis (Scheme I); k = the first-order rate constant for absorption of warfarin (hours^{-1}); k_I refers to the value obtained by one-compartment analysis (Scheme I); and k_{II} refers to the value obtained by two-compartment analysis (Scheme II). K_1 and K_{-1} are first-order distribution-rate constants (hours^{-1}) (Scheme II); K_2 = first-order rate constant for elimination of warfarin by all processes (hours^{-1}) (Scheme II); V_1 = volume of the inner compartment of which plasma is considered representative (liters) (Scheme II); and C_1 = concentration of warfarin (acid) in the plasma and inner compartment at time t (micrograms/milliliter) (Scheme II).

$$\alpha = {}^1/_2[(K_1 + K_{-1} + K_2) + \sqrt{(K_1 + K_{-1} + K_2)^2 - 4K_{-1}K_2}] \text{ (hours}^{-1}\text{)} \quad \text{(Eq. 2)}$$

$$\beta = {}^1/_2[(K_1 + K_{-1} + K_2) - \sqrt{(K_1 + K_{-1} + K_2)^2 - 4K_{-1}K_2}] \text{ (hours}^{-1}\text{)} \quad \text{(Eq. 3)}$$

For Scheme II, it may be shown that:

$$C_1 = \frac{kFD}{V_1}\left[\left\{\frac{K_{-1} - \alpha}{(k - \alpha)(\beta - \alpha)}\right\} e^{-\alpha t} + \left\{\frac{K_{-1} - \beta}{(k - \beta)(\alpha - \beta)}\right\} e^{-\beta t} + \left\{\frac{K_{-1} - k}{(\alpha - k)(\beta - k)}\right\} e^{-kt}\right] \quad \text{(Eq. 4)}$$

It may be shown that following oral administration of warfarin:

$$FD = V_1K_2A = V_d\beta A = V_d\left(\frac{0.693A}{T_{1/2}}\right) \quad \text{(Eq. 5)}$$

If both sides of Eq. 5 are divided by W, then rearrangement yields

$$\frac{0.693A}{(T_{1/2})(D/W)} = \frac{F}{(V_d/W)} \quad \text{(Eq. 6)}$$

where D/W is the milligrams/kilogram dose, and V_d/W is the apparent volume of distribution of warfarin expressed as a fraction of body weight. Equation 6 provides a pharmacokinetic interpretation of the "normalized area."

Statistical treatment of data included the following. Individual subject values of C at each sampling time, peak, area 0–24 hr., area 0–96 hr., A, normalized A value, time of occurrence of peak plasma concentration, and $T_{1/2}$ were analyzed by analysis of variance for crossover design using the data collected in each study separately. The significance levels shown in the tables opposite treatment averages are those obtained by an F-test using the among or between treatment mean square and the residual mean square in each analysis of variance. Four subjects were common to both studies; hence, there were five $T_{1/2}$ values estimated for each of these four subjects directly from their observed terminal plasma concentrations. Correlation coefficients were obtained for each subject when these $T_{1/2}$ values were correlated with the dose number, the dosing day number, and the dose of warfarin administered. All possible correlation coefficients were calculated for the correlations of peak, A, and the normalized areas with D/W, D, $1/W$, and peak; when N = 56, the values obtained with Treatments A (five 5-mg.), A (two 5-mg.), C (two 5-mg.), and D (two 5-mg.) were included, plus those from literature (11) obtained after a much higher dose; when N = 18, only those values obtained with Treatments A (five 5-mg.) and A (two 5-mg.) were included.

Pharmacokinetic analysis of the data included: (*a*) calculation of the normalized areas and interpretation by means of Eq. 6, and (*b*) fitting of some individual subject and average plasma concentrations observed following each treatment to the equations appropriate to Schemes I and II[4]. In each fitting, the plasma concentrations and corresponding time values, initial estimates of the parameters obtained graphically, the appropriate equations[4], and the program NONLIN were appropriately fed to an IBM 360/67 digital computer. The output of the computer was the least-squares estimates of the parameters, their standard deviations, and confidence intervals.

The rate of availability of warfarin (*i.e.*, rate of absorption) is indicated by the k_{II} values or by the half-absorption times [(0.693)(60)/k_{II}] in minutes. To relate the data obtained in both clinical studies with *in vitro* data, two approaches were used. The first approach made no assumption about efficiency of absorption and utilized Eq. 7:

$$\frac{\text{milligrams of warfarin (acid) absorbed in 1 hr.}}{V_d \text{ expressed as a fraction of body weight}} = \left(\frac{F}{V_d/W}\right)(D)[1 - e^{-k_{II}}] \quad \text{(Eq. 7)}$$

The second approach assumed that F has a value of unity (*i.e.*, entire dose absorbed) following Treatments A (five 5-mg.), A (two 5-mg.), C (two 5-mg.), and D (two 5-mg.); following Treatment B (one 25-mg.), F was assigned a value of 0.806[5]. Equation 8 was then utilized to obtain the *in vivo* parameter:

$$\text{milligrams of warfarin absorbed in 1 hr.} = FD[1 - e^{-k_{II}}] \quad \text{(Eq. 8)}$$

Bioavailability of warfarin (*i.e.*, relative absorption efficiency) was assessed within each study by two methods. First, normalized areas were compared by analyses of variance for crossover design. Equation 6 indicates this is equivalent to a comparison of $F/(V_d/W)$ values. If one assumes that the average V_d/W remained constant for the same panel given two or three treatments, then the difference between or among normalized areas theoretically yields the differences between or among F values. Also if the difference between two average normalized areas is significant, then the ratio of the normalized areas theoretically is equivalent to the ratio of F values following the two treatments. Secondly, V_1/F values, estimated by two-compartment analysis, were compared, taking cognizance of their 95% confidence intervals. If the assumption is made that the average V_1 remains constant for each treatment for a given panel of subjects, then the comparison of these values reflects significance or nonsignificance of differences in F values following two or more treatments.

[4] For Scheme I, the equation was $C = C^0\,(k_I/k_I - K)[e^{-Kt} - e^{-K_I t}]$. For Scheme II, Eqs. 2, 3, and 4 were used.

[5] $F_B = (D_A/D_B)\cdot(\beta_B/\beta_A)\cdot(A_B/A_A) = (22.7/23.9)\ (0.0187/0.0187)\ (140.9/165.9) = 0.806$, where the subscripts refer to Tablets A and B, and the β and A values were those estimated by nonlinear fitting to the two-compartment open model with first-order absorption.

Table III—Plasma Warfarin Concentrations[a] (Micrograms/Milliliter) Measured in Study No. 2 following 10-mg. Doses of Either Warfarin Sodium or Warfarin Potassium as Two 5-mg. Tablets

Subject	Tablets	Body Weight, kg.	Hours 1	4	8	12	24	48	72	96
1	A	72.6	1.08	0.81	0.78	0.79	0.63	0.37	0.30[b]	0.25
	C		0.86	1.54	1.02	1.09	0.96	0.77	0.58	0.46[c]
	D		0.08	1.04	0.81	0.83	0.60	0.46	0.35	0.25
2	A	59.0	1.33	1.26	0.98	0.93	0.68	0.44	0.36	0.23
	C		1.68	1.03	1.04	1.03	0.79	0.63	0.41	0.31
	D		0.34	1.68	1.13	1.03	0.87	0.56	0.40	0.26
3	A	89.4	1.02	1.00	0.66	0.66	0.61	0.46	0.33	0.27
	C		1.56	1.40	1.29	1.24	0.69	0.35	0.26	0.15[d]
	D		0.46	0.93	0.76	0.76	0.64	0.47	0.34	0.21
4	A	65.8	0.91	1.12	0.98	0.85	0.72	0.54	0.36	0.28
	C		0.47	0.87	1.01	0.93	0.77	0.53	0.45[e]	0.31
	D		0.46	1.17	1.02	0.93	0.73	0.56	0.39	0.29
5	A	73.5	0.74	0.91	0.81	0.72	0.54	0.46	0.31	0.23
	C		0.98	0.99	0.78	0.66	0.53	0.35	0.24	0.08
	D		0.70	0.93	0.82	0.86	0.66	0.49	0.40	0.27
6	A	77.1	1.06	0.95	0.76	0.89	0.60	0.36	0.25	0.20
	C		0.28	0.89	0.80	0.63	0.47	0.37	0.23	0.19
	D		0.30	1.10	0.93	0.90	0.74	0.55	0.40	0.31
7	A	91.6	0.56	1.00	0.85	0.79	0.63	0.43	0.33	0.22
	C		0.84	0.83	0.64	0.64	0.46	0.31	0.26	0.19
	D		0.24	0.89	0.82	0.74	0.62	0.45	0.33	0.24
8	A	68.0	1.52	1.17	0.89	0.78	0.69	0.45	0.28	0.13
	C		0.60	0.95	0.73	0.80	0.57	0.36	0.26	0.18
	D		0.88	0.98	0.93	0.87	0.66	0.51	0.33	0.22
9	A	68.0	0.95	1.30	0.93	0.79	0.69	0.55	0.37	0.27
	C		1.15	1.32	1.00	0.91	0.73	0.60	0.41	0.31
	D		1.14	1.14	0.93	0.81	0.59	0.43	0.37	0.23
10	A	74.8	1.16	0.86	0.86	0 77	0.57	0.36	0.30	0.20
	C		0.63	1.13	0.91	0.81	0.68	0.49	0.40	0.29
	D		0.62	0.89	0.87	0.69	0.59	0.43	0.36	0.26
11	A	93.9	0.95	0.87	0.75	0.74	0.56	0.36	0.28	0.18
	C		0.74	0.82	0.65	0.57	0.48	0.32	0.19	0.11
	D		0.41	1.00	0.76	0.74	0.55	0.41	0.30	0.25
12	A	60.3	2.01	1.68	1.58	1.15	0.83	0.67	0.31	0.21
	C		0.56	1.74	1.18	1.27	0.80	0.68	0.58	0.41
	D		1.29	1.45	1.61	1.08	0.97	0.61	0.49	0.35

[a] All zero-hour plasmas were zero. [b,c,d,e] Samples were hemolyzed and could not be assayed. [b] Value estimated from semilogarithmic plot of 48- and 96-hr. plasma concentrations. [c] Value estimated from semilogarithmic plot of 12–72-hr. plasma concentrations. [d] Value estimated from semilogarithmic plot of 24–72-hr. plasma concentrations. [e] Value estimated from semilogarithmic plot of 48–96-hr. plasma concentrations.

RESULTS

Plasma Concentration—The plasma concentrations of warfarin measured in Study No. 1 are shown in Table II, while those measured in Study No. 2 are shown in Table III. With respect to the data shown in Table II, it was shown previously (7) that a plot of the *E versus T* assay values gave a plot with a least-squares line forced through the origin having a slope of 1.00. It was concluded (7) that both methods of assay measured only unchanged warfarin following single doses of drug at the dose levels studied. Hence, in Study No. 2, only the extraction (*E*) assays were performed. The quality control chart for the assays performed for Study No. 2 is shown as Fig. 1. This gives the net absorbance/concentration ratio, observed each day assays were performed, for spiked blood bank plasma. The mean value of 0.315 agrees very closely with the factor 0.320 used in calculating the plasma concentration. The latter value was based on extensive calibration data.

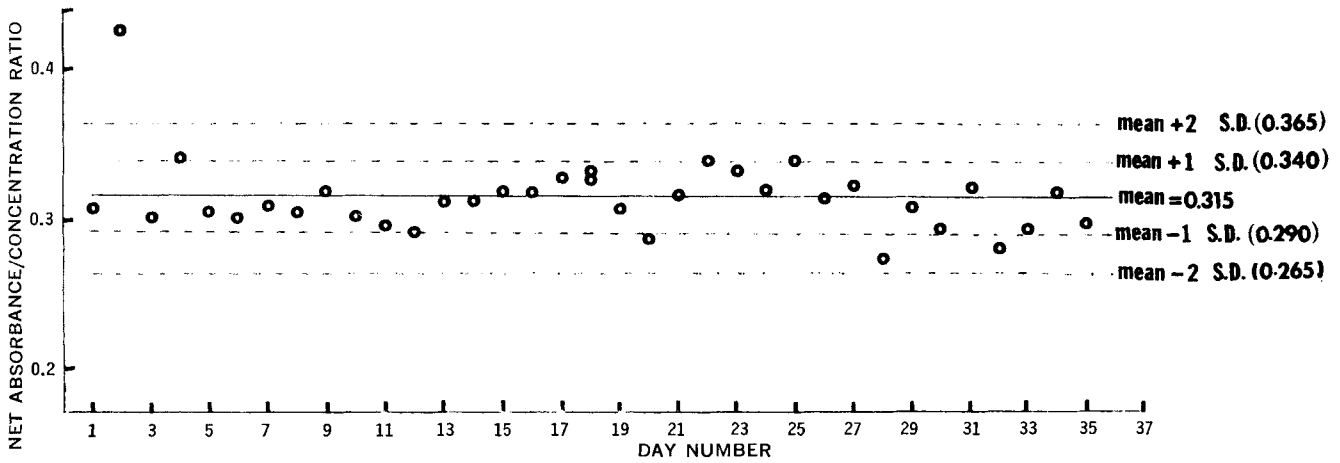


Figure 1—*Quality control chart for warfarin Study No. 2 based on spiked blood bank plasma and blood bank plasma carried through the assay along with subjects' plasmas each day assays were performed.*

Table IV—Summary of Averages for Study No. 1

Plasma Concentration, mcg./ml.	Analytical Method	Treatment: A (five 5-mg.) Average	A (five 5-mg.) CV, %[a]	B (one 25-mg.) Average	B (one 25-mg.) CV, %	ANOVA Results	Average B/ Average A × 100
At 1 hr.	*E*[b]	2.34	22.1	1.34	78.8	Sig. ($0.05 > p > 0.025$)	57
	T[c]	2.48	18.7	1.25	83.9	Sig. ($0.05 > p > 0.025$)	50
At 4 hr.	*E*	3.17	20.9	2.05	50.2	Borderline ($p \simeq 0.05$)	65
	T	3.14	24.0	2.06	54.1	Sig. ($0.025 > p > 0.01$)	66
At 8 hr.	*E*	2.71	18.7	2.21	24.7	Sig. ($0.05 > p > 0.025$)	81
	T	2.57	13.1	2.15	29.1	Borderline ($p = 0.54$)	84
At 12 hr.	*E*	2.53	20.5	2.26	19.8	Sig. ($0.025 > p > 0.01$)	89
	T	2.59	20.1	2.24	28.7	Sig. ($0.01 > p > 0.05$)	86
At 24 hr.	*E*	1.96	22.7	1.72	20.9	N.S. ($0.10 > p > 0.05$)	88
	T	1.94	19.9	1.59	26.4	Sig. ($0.05 > p > 0.025$)	82
At 48 hr.	*E*	1.31	20.0	1.11	24.5	N.S.	85
	T	1.23	19.8	1.12	25.9	N.S.	91
At 72 hr.	*E*	0.78	20.9	0.72	29.1	N.S.	92
	T	0.71	25.3	0.69	24.8	N.S.	97
At 96 hr.	*E*	0.53	37.0	0.44	41.1	N.S.	83
	T	0.53	19.8	0.43	24.9	N.S.	81
Peak plasma concentration, mcg./ml.	*E*	3.19	20.3	2.47	29.0	Sig. ($0.025 > p > 0.01$)	77
	T	3.21	21.9	2.51	31.9	Sig. ($0.005 > p > 0.001$)	78
Time of occurrence of peak plasma concentration, hr.	*E*	6.3	35.7	7.3	53.7	Borderline ($p \simeq 0.07$)	—
	T	3.0	38.4	8.0	54.8	Borderline ($p \simeq 0.07$)	—
Area 0–24 hr. mcg./ml. × hr.	*E*	58.6	17.8	47.1	25.5	Sig. ($0.025 > p > 0.01$)	80
	T	58.7	15.7	45.8	30.7	Sig. ($0.025 > p > 0.01$)	78
Area 0–96 hr. mcg./ml. × hr.	*E*	138.6	15.5	117.0	21.5	N.S.	84
	T	134.9	16.4	113.6	26.1	N.S.	84
A (estimated area 0–∞) mcg./ml. × hr.	*E*	165.4	17.5	144.9	22.3	N.S.	88
	T	157.7	16.9	135.8	24.6	N.S.	86
$T_{1/2}$, hr.	*E*	35.4	23.6	39.4	33.4	N.S.	—
	T	35.0	26.2	35.4	15.9	N.S.	—
$\frac{0.693A}{(T)_{1/2} \times (D/W)}$	*E*	9.90	11.5	7.52	16.6	Sig. ($0.01 > p > 0.001$)[d]	76
	T	9.61	9.62	7.58	18.0	Sig. ($0.025 > p > 0.01$)	79

[a] *CV* (%) = standard deviation/average × 100. [b] *E* = modified O'Reilly extraction method. [c] *T* = TLC method. [d] Result obtained by both paired *t*-test and ordinary *t*-test as well as ANOVA.

Table IV summarizes treatment averages for Study No. 1. Absorption of warfarin was slower and less efficient following Treatment B (one 25-mg.) than following Treatment A (five 5-mg.). This is of particular interest since both tablets were made by the same manufacturer. There were significant differences between treatment averages for plasma concentrations measured at 1, 4, 8, and 12 hr.; peak plasma concentrations; areas 0–24 hr., and normalized areas. For each measurement or parameter, the average for Treatment B (one 25-mg.) expressed as a percentage of the average for Treatment A (five 5-mg.) is shown in the last column of Table IV. The poorer tablet, B, gave higher coefficients of variation, in general, than the better tablet, A. This is particularly noticeable for plasma concentrations measured during the absorption phase and for the normalized areas reflecting relative absorption.

Table V summarizes treatment averages for Study No. 2. There were significant differences among treatment averages for plasma concentrations at 1 hr. and for time of occurrence of peak plasma concentrations. There were no significant differences among treatment averages for plasma concentrations measured at 4, 8, 12, 24, 48, 72, and 96 hr.; area 0–24 hr.; area 0–96 hr., *A*; normalized area; and $T_{1/2}$. The order for average plasma concentrations measured at 1 hr. was Tablet A > Tablet C > Tablet D; the order for time of occurrence of peak plasma concentrations was Tablet A < Tablet C < Tablet D.

Estimates of Bioavailability—Table VI summarizes average values of the normlized areas for all subjects in each study and for the four subjects common to both studies and the results of statistical analyses. The four subjects common to both studies gave average normalized area values very similar to the corresponding averages given by all subjects. There were no significant differences among normalized area treatment averages for all 12 subjects in Study No. 2, indicating equal bioavailability for Tablets A, C, and D following the two-tablet dose of each. By both assay methods, the differences between normalized area treatment averages for Tablets A and B were highly significant at the 25-mg. dose level in Study No. 1. The ratio of the average normalized area for Treatment B (one 25-mg.) to the average normalized area for Treatment A (five 5-mg.) was 0.84 for the *E* assays and 0.79 for the *T* assays. These values are theoretically equivalent to F_B/F_A, where the subscripts refer to the tablets. The same ratio obtained by two-compartment analysis of average plasma concentration data was 0.806 (see *Footnote 5*). Hence, absorption of warfarin following Tablet B was about 80% of absorption of warfarin following Tablet A at the 25-mg. dose level.

The results of the nonlinear least-squares fitting of average plasma concentrations following each of the five treatments is shown in Table VII (one-compartment analysis) and Table VIII (two-compartment analysis). The measures of fit, r^2 and Corr., indicate better fits to the two-compartment model than to the one-compartment model. Also, there were distinct trends in areas of poor fit in the case of the one-compartment analysis. The 95% confidence intervals of V_1/F were 6.64–7.32, 5.53–7.45, and 5.12–7.57 l. for Treatments A (two 5-mg.), C (two 5-mg.), and D (two 5-mg.), respectively. These confidence intervals overlap. This result indicates equal bioavailability of warfarin from Tablets A, C, and D.

Estimates of Absorption Rate—The k_{11} values in Table VIII are the estimated first-order rate constants for absorption of warfarin following the various treatments. The larger this number, the more rapid was the absorption. The order of absorption rates were: Tablet A > Tablet B in Study No. 1, and Tablet A > Tablet C > Tablet D in Study No. 2. The half-absorption times are summarized in Table XIV.

Variability of $T_{1/2}$—There were marked intersubject and intrasubject variations in $T_{1/2}$ of warfarin. The *average* $T_{1/2}$ of 35.4 and 39.4 hr. following Treatments A (five 5-mg.) and B (one 25-mg.), respectively, in Study No. 1 were not significantly different. Similarly, the *average* $T_{1/2}$ of 54.7, 53.1, and 53.8 hr. following Treatments A (two 5-mg.), C (two 5-mg.), and D (two 5-mg.), respectively, in Study No. 2 were not significantly different. However, the same subject administered warfarin at different times exhibited different $T_{1/2}$ values. The standard deviations caleulated from the mean squares for among subjects, among time periods, and among

Table V—Summary of Averages for Study No. 2

Plasma Concentration, mcg./ml.	Treatment: A (two 5-mg.) Average	A (two 5-mg.) *CV*, %[a]	C (two 5-mg.) Average	C (two 5-mg.) *CV*, %	D (two 5-mg.) Average	D (two 5-mg.) *CV*, %	ANOVA Results
At 1 hr.	1.11	34.1	0.86	49.1	0.58	63.7	Sig. ($0.01 > p > 0.005$)[b]
At 4 hr.	1.08	23.0	1.13	27.1	1.10	21.9	N.S. ($p > 0.25$)
At 8 hr.	0.90	25.9	0.92	22.3	0.95	24.7	N.S. ($p > 0.25$)
At 12 hr.	0.82	15.4	0.88	27.1	0.85	13.9	N.S. ($p > 0.25$)
At 24 hr.	0.65	12.6	0.66	24.1	0.68	18.2	N.S. ($p > 0.25$)
At 48 hr.	0.45	20.8	0.48	33.1	0.49	12.9	N.S. ($p > 0.25$)
At 72 hr.	0.31	11.6	0.36	38.0	0.37	13.3	N.S. ($0.25 > p > 0.10$)
At 96 hr.	0.22	19.3	0.25	47.3	0.26	15.1	N.S. ($p > 0.25$)
Peak plasma concentration, mcg./ml.	1.20	25.6	1.21	28.5	1.11	23.8	N.S. ($p > 0.25$)
Time of occurrence of peak plasma concentration, hr.	2.3	64.4	3.6	53.6	4.1	37.0	Sig. ($0.05 > p > 0.01$)
Area 0–24 hr. mcg./ml. × hr.	20.0	18.6	20.4	23.6	19.7	18.4	N.S. ($p > 0.25$)
Area 0–96 hr. mcg./ml. × hr.	48.9	14.3	51.4	27.5	51.9	15.2	N.S. ($p > 0.25$)
A (estimated area 0–∞) mcg./ml. × hr.	67.1	12.0	72.7	36.6	72.7	15.4	N.S. ($p > 0.25$)
$T_{1/2}$, hr.	54.7	24.3	53.1	26.4	53.8	17.9	N.S. ($p > 0.25$)
$\frac{0.693A}{(T_{1/2}) \times (D/W)}$	7.15	18.5	7.82	23.8	7.46	17.4	N.S. ($p > 0.25$)

[a] CV (%) = standard deviation/average × 100. [b] Multiple-range tests indicated that the average for Treatment D was significantly different from the averages for Treatments A and C but that the averages for Treatments C and D were not significantly different.

treatments were 14.1, 14.9, and 2.7 hr., respectively, using mean squares from the analysis of variance of $T_{1/2}$ in Study No. 2. This indicates that intrasubject variation of $T_{1/2}$ was of the same order of magnitude as intersubject variation of $T_{1/2}$.

This variation in $T_{1/2}$ *cannot* be explained by continued absorption of warfarin when the $T_{1/2}$ was estimated. The average $T_{1/2}$'s for treatments *within each study* are essentially equal, yet the absorption rates vary widely from treatment to treatment within each study. The average $T_{1/2}$'s after the 25-mg. label doses are *lower* than the average $T_{1/2}$'s after the 10-mg. doses. The 25-mg. doses were absorbed over longer time periods, in general, than the 10-mg. doses. If absorption was continuing when $T_{1/2}$ was estimated, one would expect the average $T_{1/2}$ to be *higher*, not lower, after 25-mg. doses than after 10-mg. doses. The $T_{1/2}$ was usually estimated from plasma concentrations observed in either the 48–96- or 24–96-hr. range; due to the rapid absorption of warfarin, absorption is complete long before 24–48 hr.

Table IX lists the $T_{1/2}$ values estimated for the four subjects common to both studies. In general, these data suggest random variation of $T_{1/2}$ for each subject from one time of administration to the next. Such variation is probably caused by changing activity of microsomal metabolizing enzymes (which change K_2) and changes in the distribution-rate constants (K_{-1} and K_1) from one administration to the next. This follows since the $T_{1/2}$'s estimated are essentially identical to $0.693/\beta$ and β is a function of all three of these rate constants, as indicated by Eq. 3.

Correlation coefficients were calculated from the data for each subject in Table IX as follows: the correlation of $T_{1/2}$ with dose number, the correlation of $T_{1/2}$ with dosing day number, and the correlation of $T_{1/2}$ with dose. All but one of the correlation coefficients were not significant ($p > 0.05$); most of the r values were not as large as the critical r for the $p = 0.10$ level. Subject B gave a significant correlation for $T_{1/2}$ with dose ($r = 0.926$; $0.05 > p > 0.02$). Overall, however, there is little statistical evidence of a significant trend linking change in $T_{1/2}$ of warfarin with either time or dose in the range studied.

Correlations of Parameters—Table X summarizes the correlation coefficients and significance levels for all possible correlations of peak, A, and $(0.693A)/T_{1/2}$ with D/W, D, $1/W$, and peak. The correlations of $(0.693A)/T_{1/2}$ with $1/W$ support a correction for body weight such as is made in Table VI. It may also be seen that $(0.693A)/T_{1/2}$ correlates better with D/W (*i.e.*, milligrams/kilogram

Table VI—Comparison of Average Normalized Areas

Study	Treatment	Number of Subjects	Average Dose of Warfarin, mg.	Assay	$0.693A/(T_{1/2})(D/W)$: All Subjects Average	All Subjects *SD*	Four Subjects Common to Studies No. 1 and 2 Average	Four Subjects Common to Studies No. 1 and 2 *SD*
No. 2, this report	A (two 5-mg.)	12	9.07	*E*[a]	7.15	1.32	6.93	1.56
	C (two 5-mg.)	12	8.93	*E*	7.82	1.86	8.29	2.64
	D (two 5-mg.)	12	9.40	*E*	7.46	1.30	8.61	1.55
					Av. 7.48		Av. 7.94	
No. 1, this report	A (five 5-mg.)	6	22.7	*E*	9.90	1.14	9.92	1.20
				T[b]	9.61	0.93	9.77	0.89
	B (one 25-mg.)	6	23.9	*E*	7.52	1.25	7.88	1.40
				T	7.58	1.36	7.46	1.49
O'Reilly *et al.*	1.5 mg. sodium warfarin/kg.	14	93.3[c]	*E*	8.71	1.70		
Weighted average	*E* assays only, without B (one 25-mg.)				8.04			

[a] E = simple extraction assay. [b] T = TLC assay. [c] The 1.5 mg. sodium warfarin/kg. body weight (average dose of 100 mg. sodium warfarin) is equivalent to 1.4 mg. warfarin acid/kg. body weight (average dose of 93.3 mg. warfarin acid). [d] No significant difference among averages ($p > 0.25$). [e] Significant difference among averages ($p < 0.001$). [f] Significant difference between averages ($0.01 > p > 0.001$). [g] Significant difference between averages ($0.025 > p > 0.01$). [h] Significant difference between averages ($0.02 > p > 0.01$). [i] Significant difference between averages ($0.05 > p > 0.02$).

Table VII—Parameters Estimated by Nonlinear Least-Squares Fitting of Average Plasma Concentrations (Extraction Assay), Their Standard Deviations, and Two Measures of Closeness of Fit. Fitting Was to the One-Compartment Open Model with First-Order Absorption

Estimated Parameter	Treatment				
	Study No. 1		Study No. 2		
	A (five 5-mg.)	B (one 25-mg.)	A (two 5-mg.)	C (two 5-mg.)	D (two 5-mg.)
k_1, hr.$^{-1}$	1.34 (0.124)	0.695 (0.0994)	2.55 (0.494)	1.45 (0.193)	0.680 (0.135)
K, hr.$^{-1}$	0.0198 (0.00108)	0.0174 (0.00161)	0.0168 (0.00257)	0.0168 (0.00168)	0.0163 (0.00233)
C^0, mcg./ml.[a]	3.23[b] (0.0696)	2.51[b] (0.0941)	1.07 (0.06161)	1.09 (0.0448)	1.10 (0.0644)
Measures of fit					
r^2	0.999	0.996	0.994	0.996	0.994
Corr.	0.997	0.993	0.981	0.984	0.976

[a] $C^0 = F \cdot D/V_d$. [b] $F_B = D_A C^0_B / D_B C_A = (22.7)(2.51)/(23.9)(3.23) = 0.74$, where the subscripts refer to Tablets A and B. Calculation assumes Vd constant for both treatments and $F_A = 1$.

dose) than does A (area $0 \rightarrow \infty$). This supports the calculation of normalized areas to estimate bioavailability.

Regressions (not reported) also indicate excellent agreement between data of this study and that of O'Reilly *et al.* (11). For example, using the regression equations for peak on D/W and $(0.693A)/T_{1/2}$ on D/W, derived from the 18 points resulting from Treatments A (five 5-mg.) and A (two 5-mg.), one can estimate expected values of peak and $(0.693A)/T_{1/2}$ after a 1.5-mg./kg. dose of sodium warfarin by extrapolation; these estimated values agreed very closely with the average peak and $(0.693A)/T_{1/2}$ values calculated from the data of O'Reilly *et al.* (11). This also suggests that warfarin kinetics are linear and *not* dose dependent in man in the range of 10–100-mg. doses of sodium warfarin. A future paper (12) also supports this and the applicability of the two-compartment open model to warfarin administered intravenously.

Tablet Potencies—Tablet potencies obtained by official assay of tablets are given in Table XI. These drugs are labeled in terms of their sodium or potassium salt contents. All tablets were within acceptable limits. Since the plasma concentrations were expressed in terms of warfarin (acid), the dose values used in the various calculations were based on 4.54, 23.9, 4.47, and 4.70 mg. of warfarin (acid) per tablet for Tablets A, B, C, and D, respectively. Use of such values for individual subjects would be valid if there were not large tablet-to-tablet variations. Here, five tablets of each lot were individually assayed. The results, also shown in Table XI, indicate that tablet-to-tablet variability in warfarin content was quite small in the samples studied. Hence, use of the average assay value to estimate dosage for the individual subject appears to be justified in this case.

Solubility of Warfarin in 0.1 *N* Hydrochloric Acid—At room temperature, the solubility measured was 4.35 (*SD* 0.25) mcg. warfarin (acid)/ml. At 37°, the solubility measured was 7.36 (*SD* 0.49) mcg. warfarin (acid)/ml. Hence, during exposure to the 900 ml. of 0.1 *N* hydrochloric acid in the dissolution-rate studies, a maximum of 6.62 mg. (*SD* 0.41) of warfarin (acid) can be in solution. Since one 5-mg. tablet of sodium warfarin contains 4.665 mg. of warfarin (acid), the solution would be 70% saturated with warfarin if the tablet completely dissolved during the 30-min. exposure time

Table VIII—Parameters Estimated by Nonlinear Least-Squares Fitting of Average Plasma Concentrations (Extraction Assay), Their Standard Deviations, and Two Measures of Closeness of Fit and Several Other Parameters Calculated from Them. Fitting Was to Two-Compartment Open Model with First-Order Absorption

Estimated Parameter	Treatment				
	Study No. 1		Study No. 2		
	A (five 5-mg.)	B (one 25-mg.)[a]	A (two 5-mg.)	C (two 5-mg.)	D (two 5-mg.)
k_{11}, hr.$^{-1}$	0.836 (0.0741)[b]	0.450	2.19 (0.129)	1.06 (0.102)	0.531 (0.0545)
K_1, hr.$^{-1}$	0.132 (0.0201)	0.132	0.0412 (0.0028)	0.0554 (0.0093)	0.0673 (0.0115)
K_{-1}, hr.$^{-1}$	0.315 (0.0206)	0.315	0.116 (0.0030)	0.140 (0.0102)	0.116 (0.0075)
K_2, hr.$^{-1}$	0.0270 (0.0016)	0.0270	0.0206 (0.0004)	0.0195 (0.0010)	0.0201 (0.0012)
V_1/F, liters	5.065 (0.300)	6.283	6.98 (0.108)	6.49 (0.301)	6.35 (0.386)
Measures of fit					
r^2[c]	1.000	0.992	1.000	1.000	1.000
Corr.[d]	1.000	0.983	1.000	0.998	0.998
Other parameters					
α, hr.$^{-1}$	0.455	0.455	0.164	0.201	0.191
β, hr.$^{-1}$	0.0187	0.0187	0.0144	0.0136	0.0122
$T_{1/2} = \frac{0.693}{\beta}$, hr.	37.1 [38.0][e]	37.1 [36.8]	48.1 [45.9]	50.9 [52.0]	56.8 [52.5]
$\frac{V_d}{F} = \frac{V_1}{F}\frac{\alpha}{K_{-1}}$, liters	7.29	9.08	9.84	9.35	10.48
$\frac{F}{V_d/W}$	9.52 [9.90][f]	7.61 [7.52]	7.57 [7.15]	7.97 [7.82]	7.11 [7.46]

[a] Plasma concentrations fitted holding K_1, K_{-1}, and K_2 constant and same values as Treatment A (five 5-mg.). [b] Numbers in parentheses are standard deviations of estimated parameters. [c] $r^2 = (\Sigma_{obs.}{}^2 - \Sigma_{dev.}{}^2)/\Sigma_{obs.}{}^2$. [d] Corr. is correlation coefficient for regression of predicted and observed plasma concentrations. [e] Numbers in square brackets are half-lives calculated directly from *average* terminal plasma concentrations and are included for comparison purposes. These numbers are not the same as those in Tables IV and V, which are average half-lives of individual subjects. [f] Numbers in square brackets are treatment averages calculated from individual subject's data directly, using the formula $0.693A/(T_{1/2})(D/W)$.

Table IX—Warfarin Half-Lives Estimated from Terminal Plasma Concentrations for Four Subjects Common to Both Clinical Studies

Subject	Subject Number in Study No. 1	Subject Number in Study No. 2	Half-Life, hr. Study No. 1 Phase I	Study No. 1 Phase II	Study No. 2 Phase I	Study No. 2 Phase II	Study No. 2 Phase III	Average
B	4	2	26.2[b]	33.7[a]	51.3[c]	49.9[d]	42.0[e]	40.0
Cr	3	3	47.2[a]	55.0[b]	59.9[c]	34.1[d]	34.5	46.1
Ch	5	6	56.9[b]	31.1[a]	52.1[d]	56.8[e]	74.5[c]	54.3
R	1	12	28.8[a]	34.3[b]	59.9[e]	42.7[c]	76.8[d]	48.5
Dose number			1	2	3	4	5	
Dosing day number			1	22	64	85	106	

[a] Treatment A (five 5-mg.). [b] Treatment B (one 25-mg.). [c] Treatment A (two 5-mg.). [d] Treatment C (two 5-mg.). [e] Treatment D (two 5-mg.).

Table X—Correlation Coefficients (r) and Significance Levels (p) for Pairs of Parameters

		N	Ordinate: Peak r	Peak p	A r	A p	$0.693A/T_{1/2}$ r	$0.693A/T_{1/2}$ p
Abscissa	D/W	56[a]	0.971	<0.001	0.932	<0.001	0.972	<0.001
		18[b]	0.970	<0.001	0.891	<0.001	0.975	<0.001
	D	56	0.969	<0.001	0.959	<0.001	0.924	<0.001
		18	0.913	<0.001	0.942	<0.001	0.940	<0.001
	$1/W$	56	0.251	$0.10 > p > 0.05$	0.227	$0.10 > p > 0.05$	0.338	$0.02 > p > 0.01$
		18	0.526	$0.02 > p > 0.01$	0.277	>0.10	0.451	$0.05 > p > 0.02$
	Peak	56	—	—	0.945	<0.001	0.959	<0.001
		18	—	—	0.910	<0.001	0.981	<0.001

[a] Includes the six subjects in this study given A (five 5-mg.); the 12 subjects in this study each given A (two 5-mg.), C (two 5-mg.), and D (two 5-mg.); plus 14 subjects given 1.5 mg./kg. (average dose 100 mg.) of sodium warfarin equivalent to 1.4 mg./kg. (average dose 93.3 mg.) of warfarin acid reported by O'Reilly *et al.* (11). [b] Includes the six subjects in this study given A (five 5-mg.) and the 12 subjects given A (two 5-mg.).

in the acid solution.

***In Vitro* Rate of Dissolution Studies and Disintegration Times**—Figure 2 is a plot of the dissolution data for Tablets A and B used in Study No. 1. The points correspond to the average percent dissolved of five tablets of each lot. The vertical bars mark off one standard deviation on either side of the average. Almost negligible amounts of warfarin dissolved from these tablets during the 30-min. exposure to 0.1 N hydrochloric acid. The average times required to dissolve 25, 50, and 75% of the warfarin from these two tablets are summarized in Table XII. The differences between the averages were highly significant in each case. Tablet A dissolved more rapidly than Tablet B by all criteria. Since readings were made at very frequent time intervals, one may also estimate the rate of dissolution from these data. A plot (not shown) of rate of dissolution of Tablet A *versus* time has a sharp peak just after 30 min. and then falls off rapidly. However, a similar plot (not shown) for Tablet B is low and flat over most of the observation period.

Figure 3 is a plot of the dissolution data for Tablets A, C, and D used in Study No. 2. The points to the far left of both Figs. 2 and 3 are the same since Tablet A was used in both studies. Again, almost negligible amounts of warfarin dissolved from all three tablets during the 30-min. exposure to 0.1 N hydrochloric acid. The average times required to dissolve 25, 50, and 75% of the warfarin from these three tablets are summarized in Table XII. The differences among the tablet averages are highly significant ($p < 0.001$) in each case as determined by analysis of variance. There were no significant differences among the trial averages in each case ($p > 0.10$). The order of rates of dissolution was Tablet A > Tablet C > Tablet D.

Table XI—Tablet Potencies as Measured by Official Assay Procedure

Aliquot of Powder from 20 Tablets	Potency (mg./Tablet)[a] Tablet A Salt[c]	Tablet A Acid[d]	Tablet B Salt[c]	Tablet B Acid[d]	Tablet C Salt[e]	Tablet C Acid[d]	Tablet D Salt[c]	Tablet D Acid[d]
1	4.84	4.51	25.9	24.2	5.02	4.47	4.94	4.61
2	4.93	4.60	25.4	23.7	4.99	4.44	5.10	4.76
3	4.82	4.50	25.5	23.8	5.05	4.50	5.08	4.74
Average	4.86	4.54[b]	25.6	23.9[b]	5.02	4.47[b]	5.04	4.70[b]
SD	0.059	0.055	0.265	0.265	0.030	0.030	0.087	0.081
Percent of label	97.2	—	102.4	—	100.4	—	100.8	—
Tablet No.								
1	4.75	4.43	24.3	22.7	4.91	4.37	4.79	4.47
2	4.81	4.49	24.4	22.8	5.00	4.45	4.91	4.58
3	4.74	4.42	24.5	22.9	4.93	4.39	4.89	4.56
4	4.89	4.56	24.7	23.0	4.97	4.42	5.07	4.73
5	4.83	4.51	24.7	23.0	4.92	4.38	4.83	4.51
Average	4.80	4.48	24.5	22.9	4.95	4.40	4.90	4.57
SD	0.061	0.058	0.179	0.130	0.038	0.033	0.107	0.099
Percent of label	96.0	—	98.0	—	99.0	—	98.0	—

[a] Ratios of molecular weights are: warfarin acid/sodium warfarin = 0.933 and warfarin acid/potassium warfarin = 0.890. [b] These averages were used to estimate milligrams/kilogram dosage, D/W, following oral administration of the various tablets. [c] As sodium warfarin. [d] As warfarin (acid). [e] As potassium warfarin.

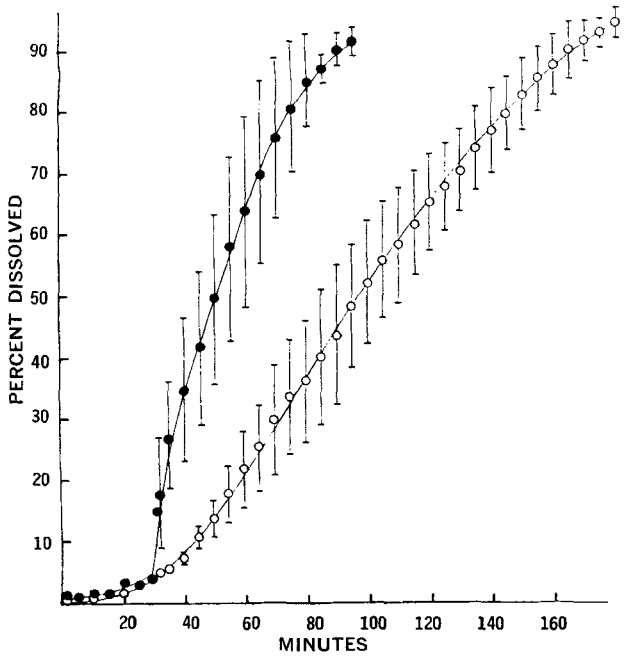


Figure 2—*Dissolution results obtained with Tablets A and B used in Study No. 1. Key: ●, Tablet A; and ○, Tablet B. Points are averages for five tablets. Bars mark off 1* SD *on either side of the average.*

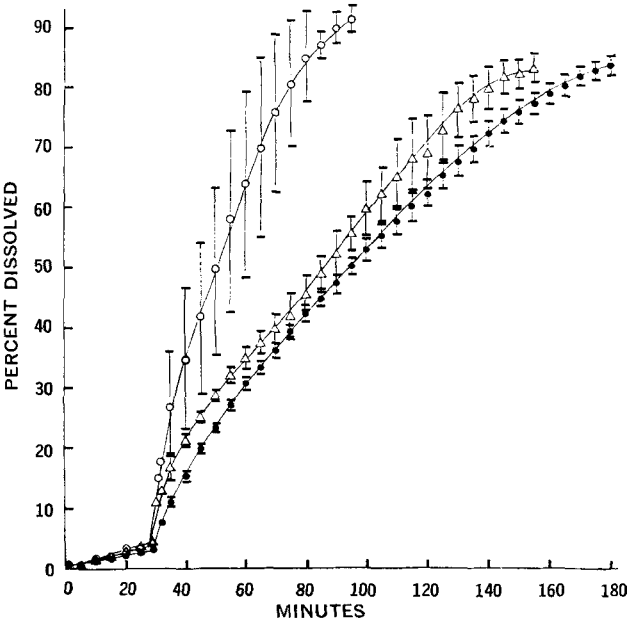


Figure 3—*Dissolution results obtained with Tablets A, C, and D used in Study No. 2. Key: ○, Tablet A; △, Tablet C; and ●, Tablet D. Points are averages for five tablets. Bars mark off 1* SD *on either side of the average.*

The disintegration times determined in water in the USP apparatus without plastic disks and the times to dissolve 50% of the warfarin ($t_{50\%}$) in the dissolution test are compared in Table XIII for the four tablets studied. Although the disintegration test distinguished between Tablets A and B, it failed to distinguish between Tablets A, C, and D. This is more evidence of the advisability of dissolution-rate criteria rather than disintegration time for quality control purposes.

The authors prepared 5-mg. sodium warfarin tablets containing 20% Veegum-F, spray-dried dextrose, and calcium stearate by direct compaction on a Stokes single-punch tablet press. These tablets released warfarin 16 times more rapidly *in vitro* than the best commercial 5-mg. tablets on the basis of $t_{50\%}$ values. These tablets also disintegrated in 0.1 *N* hydrochloric acid under static conditions in about 1 min., whereas all three commercial 5-mg. tablets remained intact in such an acid environment under similar conditions for at least 30 min. However, a 25-mg. tablet of similar composition released its warfarin only 1.3 times more rapidly than Tablet B. Further investigations are being made on an improved 25-mg. tablet; results will be published separately.

Correlation of *In Vivo* with *In Vitro* Results—The results obtained in man may be correlated in many ways with the results obtained *in vitro*. One may use the observed data directly. For example, Fig. 4 is a plot of the average warfarin plasma concentration at 1 hr. postadministration against the average amount of warfarin (acid) dissolved *in vitro* in 1 hr. To obtain the abscissa values for this plot, the average amount of warfarin (acid) dissolved per tablet *in vitro* in 1 hr. was multiplied by the number of tablets administered in the clinical study (*i.e.*, either 1, 2, or 5).

Pharmacokinetically, it is more desirable to correlate indirectly the *in vivo* with *in vitro* results. Three approaches were taken:

1. Table XIV lists the half-absorption times in man (calculated from the k_{II} values in Table VIII) and the times required to dissolve 50% of the warfarin in the dissolution test. There appears to be a dose dependency in the results achieved by this method. At the 25-mg. dose level (Study No. 1), the *in vitro* $t_{50\%}$ values almost exactly predict the half-absorption times in man. At the 10-mg. dose level (Study No. 2), there is a perfect rank order correlation but, in all cases, the half-absorption times are less than the *in vitro* $t_{50\%}$ values.

2. Figure 5 is a plot of the milligrams of warfarin (acid) absorbed in 1 hr. divided by V_d/W against the average amount of warfarin (acid) dissolved *in vitro* in 1 hr. The ordinate values for this plot were calculated using Eq. 7, the $F(V_d/W)$ values and k_{II} values listed in Table VIII, and the doses listed in Table VI. The abscissa values were obtained as already indicated. This plot makes no assumptions about efficiency of absorption (*i.e.*, the absolute amount of warfarin absorbed) from any tablet.

3. Figure 6 is a plot of estimated milligrams of warfarin (acid) absorbed in man in 1 hr. against the average amount of warfarin (acid) dissolved *in vitro* in 1 hr. The ordinate values for this plot were calculated using Eq. 8, the k_{II} values listed in Table VIII, the doses listed in Table VI, and F values of unity for Tablets A, C, and D and 0.806 for Tablet B (see *Footnote 5*). The first four points appear to be randomly distributed about a straight line; the least-squares line free to pass through any intercept has the equation: *in vivo* = 0.527 + 1.18 (*in vitro*), (r = 0.953; p = 0.05). The line forced through the ori-

Table XII—Summary of Average Times Required to Dissolve 25, 50, and 75% of the Warfarin in Tablets A, B, C, and D

Percent Dissolved	Tablet				Results of Statistical Analyses	
	A	B	C	D	A *versus* B	Among A, C, and D
25[a]	35.9	65.8	45.1	51.8	$p < 0.001$	$p < 0.001$
50[b]	51.3	96.6	86.4	94.6	$p > 0.001$	$p < 0.001$
75[c]	68.1	135.8	125.9	147.4	$p > 0.001$	$p < 0.001$

[a] Trial averages for Tablets A, C, and D were 45.1, 43.1, 45.0, 45.9, and 42.3. [b] Trial averages for Tablets A, C, and D were 81.0, 75.6, 80.2, and 70.8. [c] Trial averages for Tablets A, C, and D were 121.8, 107.5, 115.0, 114.9, and 109.8. There were no significant differences among any of the trial averages above in each set ($p > 0.10$).

Table XIII—Comparison of Disintegration Times with Time for 50% of Warfarin to Dissolve *In Vitro*

Tablet	Disintegration Time[a], min.		$t_{50\%}$[b], min.	
	Average	*SD*	Average	*SD*
A	11.0	1.18	51.3	9.39
B	20.5	4.05	96.6	13.0
C	10.7	1.15	86.8	5.66
D	11.2	0.51	94.6	2.64

[a] Determined in water in the USP apparatus without plastic disks at 37°. [b] Determined by method described in *Experimental* section of this report.

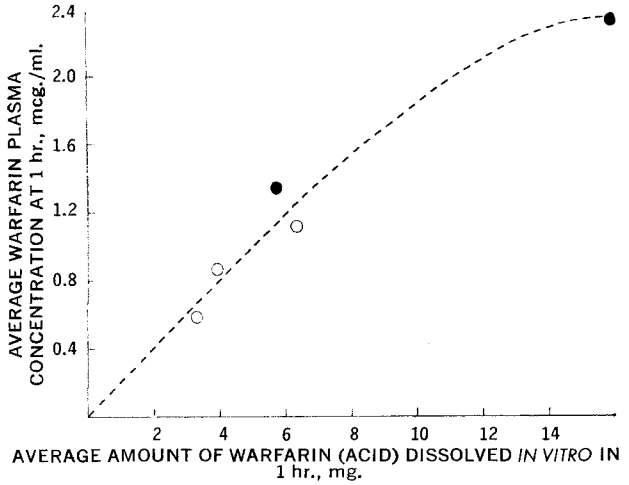


Figure 4—*Correlation of* in vivo *with* in vitro *results. Plot of average plasma concentration of warfarin at 1 hr. against average amount of warfarin dissolved* in vitro *in 1 hr. Key:* ●, *25-mg. dose;* ○, *10-mg. dose; points from left to right refer to Treatments D (two 5-mg.), C (two 5-mg.), B (one 25-mg.), A (two 5-mg.), and A (five 5-mg.).*

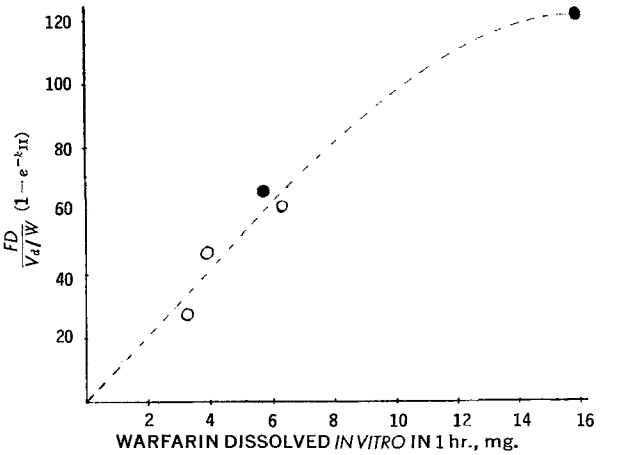


Figure 5—*Correlation of* in vivo *with* in vitro *results. Plot of* FD/(V_d/W)($1 - e^{-k_{II}}$) *against milligrams of warfarin (acid) dissolved* in vitro *in 1 hr. The ordinate is equivalent to the estimated milligrams of warfarin (acid) absorbed in 1 hr. divided by* V_d *expressed as a fraction of body weight. Key: points from left to right refer to Treatments D (two 5-mg.), C (two 5-mg.), B (one 25-mg.), A (two 5-mg.), and A (five 5-mg.).*

gin has the equation: *in vivo* = 1.27 (*in vitro*), and this is the line drawn through the points on the plot.

DISCUSSION

Normalized areas, calculated in the manner indicated in the *Experimental* section, were used for the following reasons: (*a*) In fitting individual subject sets of plasma concentrations to Eqs. 2, 3, and 4, convergence was not always obtained. Hence, the parameters could not be estimated; this also occurred with average plasma concentrations for Treatment B (one 25-mg.), which explains *Footnote* [a] of Table VIII. (*b*) Values of A estimated by the usual method (9) agreed very closely with values obtained by integration of the triexponential equation obtained by two-compartment fitting. (*c*) Values of $T_{1/2}$ estimated directly from terminal plasma concentrations agreed very closely with corresponding values of $0.693/\beta$, where β was obtained by exponential fitting to Eqs. 2, 3, and 4. Fitting of individual subject plasma concentrations, where feasible, gave similar parameters for a given treatment to those estimated from the average plasma concentrations. Parameters listed in Tables VII and VIII were obtained by assigning equal weights to the plasma concentrations in a given set. Reciprocal weighting gave similar results in these cases. Since it was shown formerly (7) that the error involved in a measured warfarin plasma concentration is independent of the magnitude of the plasma concentration in the range of concentrations reported here, it appeared to be more valid *in these cases* to use equal weights.

Some readers may question the emphasis on average peak plasma concentrations in Tables IV, V, and X. The senior author has noticed high correlations of peak with D/W and high correlations of peak with $0.693A/T_{1/2}$ in similar studies with other drugs. High correlations between these pairs of parameters were also observed in these studies (Table X) and appeared worthy of reporting. Pharmacokinetically, the peak plasma concentration is a complicated function. However, these high correlations suggest that the widespread use of peak plasma concentration by the medical profession may, in many cases, be supported by sound statistical evidence. Moreover, in Tables IV and V, the average peak plasma concentration of individual subjects is often considerably higher than the peak of the average plasma concentration curve; too frequently the latter is used rather than the former and leads to a distortion not warranted by the data.

The results indicate that absorption of warfarin in man is dissolution-rate controlled, as suggested from previous investigations (2, 3), and that such control extends down into the therapeutic dose range. Some of the commercial tablets studied have dissolution rates *in vitro*, and presumably *in vivo*, that are just in the critical range. The warfarin in Tablet B was not completely available for absorption in man, while another tablet, A, made by the same manufacturer, allowed complete bioavailability of its contained warfarin.

Tablets A, C, and D provided equal bioavailability of the warfarin in Study No. 2, but the rate constants for absorption of warfarin from these tablets at the 10-mg. dose level varied over a fourfold range. The data suggest that the *in vivo* dissolution rate of warfarin from Tablet D is just inside the critical range; if the rate was much slower, the warfarin contained in such a tablet may not be completely available to man.

It may be theorized that one would expect absorption from five 5-mg. tablets to be faster than from one 25-mg. tablet on the basis of difference in surface area of the intact tablets. This concept is most probably erroneous. First, this difference in surface area of intact tablets is almost negligible when related to the available surface when the tablets disintegrate. When a tablet disintegrates, the increase in surface area is of the order of 1000–10,000 times the surface area of the intact tablet. Second, the opposite results with capsules have already been reported in the literature. Riegelman (13) showed that average plasma levels of griseofulvin in 16 subjects were *higher* after two 250-mg. capsules than after four 125-mg. capsules. It is realized that tablets are different from capsules, but these results are worthy of repetition. Third, there is no *a priori* reason to expect that the rate of dissolution of drug from a higher potency tablet will always be less than that from a lower potency tablet. This can readily be perceived if one considers that the higher dose could be punched in a tablet with lactose and a good disintegrant while the lower dose of drug could be directly compressed without adjuvants or, even worse, encased in cement. The way the tablet is made is the important thing, and both good high and low potency tablets of almost any drug may be prepared.

The results reported here do not imply that other commercial warfarin tablets, made by the same or different manufacturers, will provide satisfactory results in man. For example, a 10-mg. tablet, made by the same manufacturer that made Tablet C, was found to have an average *in vitro* $t_{50\%}$ of 144 min.; this tablet was the slowest dissolving of six different commercial tablets tested *in vitro*. If the *in vivo–in vitro* correlations established in these studies are valid when extrapolated to lower *in vitro* rates of dissolution, one would expect absorption in man following oral administration of this 10-mg. tablet to be quite slow relative to that for two A tablets and perhaps to show a lower bioavailability than even Tablet B.

Plasma concentrations of individual subjects following intravenous administration of sodium warfarin have also been shown to be fit by the two-compartment open model (12), thus supporting the preference for this model for plasma concentrations observed after oral administration. The analysis of the intravenous data gave an average value of 0.114 for V_d/W, which has a reciprocal, $1/(V_d/W)$, of 8.77. The average value of $V_{d\ \text{extrap.}}/W$, reported by O'Reilly *et al.* (3), was 0.125, which has a reciprocal of 8.00. It is shown in the *Appendix* that, for warfarin, $V_{d\ \text{area}}$ and $V_{d\ \text{extrap.}}$ are essentially identical. When these values are substituted into Eq. 6, using either the average normalized areas of Table VI or β and A values of Table VIII, F values for Treatment A (five 5-mg.) range from 1.13–1.27. The fact that F values greater than unity are obtained by this method is incongruous

Table XIV—Half-Absorption Times of Warfarin Estimated from Average Plasma Concentrations Compared with Times Required to Dissolve 50% of Warfarin *In Vitro*

Study	Treatment	Time Required to Dissolve 50% of Warfarin *In Vitro*, min.	Half-Absorption Time in Man, min.
No. 1	A (five 5-mg.)	51.3	49.4
	B (one 25-mg.)	96.6	92.4
No. 2	A (two 5-mg.)	51.3	19.0
	C (two 5-mg.)	86.8	39.2
	D (two 5-mg.)	94.6	78.3

but does suggest strongly that the absorption of warfarin following Tablet A was essentially complete (*i.e.*, 100% absorption). Since it is unlikely that efficiency of absorption of warfarin after two A tablets in Study No. 2 would be less than after five A tablets in Study No. 1, absorption of warfarin following Tablet A in Study 2 was probably essentially complete. Since the average normalized area ratios for Treatments A (two 5-mg.), C (two 5-mg.), and D (two 5-mg.) were not significantly different in Study No. 2, absorption of warfarin following Tablets C and D was probably essentially complete in Study No. 2. However, the discrepancy between the average normalized areas for Tablet A in Studies No. 1 and 2 (Table VI) is difficult to explain. For each of the four subjects common to both studies given Treatments A (five 5-mg.) and A (two 5-mg.), the ratios $A_2/A_1 \cdot D_1/D_2$ and $(T_{1/2})_2/(T_{1/2})_1$, where the subscripts refer to the study number, were calculated. The average dose corrected area ratio was 1.10 (range 0.942–1.32), and the average half-life ratio was 1.77 (range 1.27–1.91). These results in relation to Eq. 6 indicate that for these subjects either F was lower in Study No. 2 than in Study No. 1, or that V_d/W was higher in Study No. 2 than in Study No. 1, or that both factors were operative. Since the data for these four subjects gave similar average normalized areas to the average normalized areas obtained for the entire panel, this indicates a similar hypothesis is valid for the entire panels also.

APPENDIX: VOLUMES OF DISTRIBUTION

In terms of the parameters of the two-compartment open model, various volumes of distribution were described by Riegelman *et al.* (10). From the equations given by these authors, the following equations were derived:

$$V_{d\ \text{area}} = V_1\left(\frac{\alpha}{K_{-1}}\right) \qquad \text{(Eq. 1A)}$$

$$V_{d\ \text{extrap.}} = V_1\left(\frac{\alpha - \beta}{K_{-1} - \beta}\right) \qquad \text{(Eq. 2A)}$$

$$V_{d\ \text{ss}} = V_1\left(\frac{\alpha + \beta - K_2}{K_{-1}}\right) \qquad \text{(Eq. 3A)}$$

Since for warfarin, β is much smaller than either α or K_{-1}, for warfarin the $V_{d\ \text{area}}$ is approximately the same value as $V_{d\ \text{extrap.}}$. Since the calculations in Table VI relate to $V_{d\ \text{area}}$ and the estimates of volume of distribution of warfarin made by O'Reilly *et al.* (3) following intravenous injection are really estimates of $V_{d\ \text{extrap.}}$, these are directly comparable in the case of warfarin but not necessarily for another drug. This appendix was included to validate the comparison made under *Estimates of Bioavailability* in the text.

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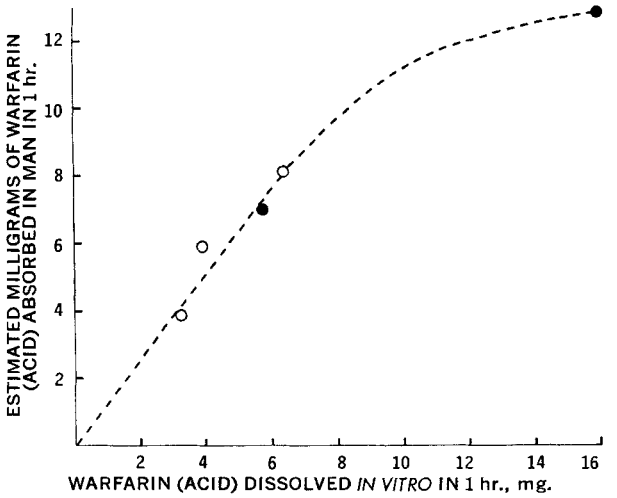

Figure 6—*Correlation of* in vivo *with* in vitro *results. Plot of estimated amount of warfarin absorbed in man in 1 hr. against amount of warfarin dissolved* in vitro *in 1 hr. Key:* ○, *10-mg. label dose; and* ●, *25-mg. label dose. Points from left to right refer to Treatments D (two 5-mg.), C (two 5-mg.), B (one 25-mg.), A (two 5-mg.), and A (five 5-mg.).*

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