Physicochemical and Physiologic Factors Affecting the Absorption of Warfarin in Man

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The relationship between the *in vitro* dissolution kinetics and the *in vivo* intestinal absorption characteristics of tablet preparations of the coumarin anticoagulant warfarin has been studied. The composition of the dissolution medium had a significant quantitative and qualitative effect on dissolution kinetics. Warfarin absorption in man varied with respect to lag time, absorption rate constants, and the type of kinetics observed. These variations may result from variable gastric emptying times and from differences in the pH of gastric fluids.

THE IMPORTANCE of pharmaceutical formulation in modifying the gastrointestinal absorption of drugs from tablets has been documented extensively (*References 1–3* and references cited therein.) To predict the *in vivo* absorption characteristics of various preparations, reliable *in vitro* testing procedures must be available. Recent studies (4, 5) have suggested that the development of such testing procedures may be feasible. Consistent absorption rate and complete physiologic availability are desired characteristics for all drugs, but these properties are particularly necessary for the coumarin anticoagulants. These agents have important therapeutic indications, *e.g.*, coronary heart disease, pulmonary embolism, and prevention of clotting after insertion of artificial heart valves. The magnitude and duration of pharmacologic activity of this class of drugs is a function of drug level in the body (6); excessively high coumarin levels can lead to hemorrhage with potentially disastrous consequences. The incidence of bleeding during anticoagulant therapy is related directly to excessive reduction of prothrombin activity (7).

The dosage form characteristics of coumarin anticoagulants can have a pronounced effect on their absorption. Lozinski (8) reported that a change in formulation of bishydroxycoumarin tablets caused such an augmentation of the therapeutic response that hemorrhage ensued in some instances. O'Reilly *et al.* (9) administered bishydroxycoumarin as tablets, powder, and solution and found appreciable differences in and correlations between the time of maximum blood levels and the onset of action. The drug was rapidly absorbed as a solution and slowly absorbed as tablets. From 10 to 30% of the dose was recovered in the feces after administration of tablets; no unchanged drug was found in the feces after intravenous administration. Warfarin is more readily and completely absorbed than bishydroxycoumarin (9). A comparison of warfarin blood levels after oral administration of 100 mg. in solution, in tablets containing the drug as the free acid, and in tablets containing sodium warfarin, showed that the most rapid absorption occurred from solution (9). Surprisingly, the free acid in tablets was as readily absorbed as the sodium salt in tablets (9).

The purpose of the present study was to compare the *in vitro* dissolution and *in vivo* absorption characteristics of different tablet preparations of warfarin and to examine the relationship between these two properties.

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EXPERIMENTAL

Absorption Study.—Normal adult volunteers received 100 mg. of warfarin (as 25-mg. tablets) orally in the morning on an empty stomach. The tablets were swallowed whole together with 250 ml. of water. Food was withheld for at least 2 hr. after drug administration. Blood specimens were obtained at frequent intervals (3 to 7 samples) during the first 6 hr., and then at 12, 24, 48, and 72 hr. after drug administration. Warfarin concentrations in plasma were determined by the method of O'Reilly *et al.* (10). The amount absorbed as a function of time was calculated by the method of Wagner and Nelson (11).

Determination of Dissolution Rate.—*In vitro* dissolution tests were carried out by the beaker method of Levy and Hayes (12). For the initial screening tests 350 ml. water was used as the dissolution medium and a stirring rate of 60 r.p.m. was employed. In subsequent tests, the dissolution medium consisted of 300 ml. of 0.01 to 0.1 *N* hydrochloric acid for the first 30 min. Fifty milliliters of tris(hydroxymethyl)aminomethane solution of sufficient concentration to yield a final pH of 7.4 was then added. A stirring rate of 50 r.p.m. was employed in these experiments. From 3 to 5 tablets were used for each test. Warfarin was determined spectrophotometrically at 270 and 306 mμ in the organic phase obtained after chloroform (5 ml.) extraction of a 3-ml. aqueous sample acidified by addition of 1 ml. of concentrated HCl.

Tablet Preparations.—The several tablet preparations tested consisted of commercially available as well as experimental formulations obtained from a pharmaceutical manufacturer. Each of the tablets contained 25 mg. of warfarin as the free acid or the sodium salt.

RESULTS AND DISCUSSION

The *in vitro* dissolution rate in water of 7 different tablet preparations was evaluated. Markedly different rates were observed; dissolution half-lives ranged from 4 to about 1400 min. Data for 4 of the preparations are shown in Fig. 1. A and C are commercial products containing sodium warfarin, B is an experimental formulation containing sodium warfarin, and D is an experimental formulation containing warfarin as the acid. Preparations A and B dissolved rapidly, C dissolved slowly, and D very slowly. Accordingly, forms A, C, and D were chosen for clinical study.

The times for 50% *in vivo* absorption (including absorption lag time) of the 3 preparations in a total of 10 tests are listed in Table I. There were marked

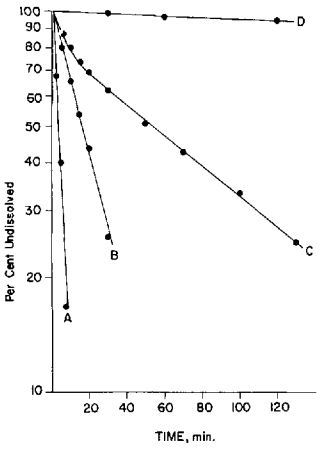

Fig. 1.—Semilogarithmic plot of per cent warfarin undissolved from several tablet formulations *vs.* time. The medium used was distilled water.

TABLE I.—TIME FOR 50% ABSORPTION[a] OF WARFARIN FROM DIFFERENT TABLET PREPARATIONS

Subject	Sex	Age	Prep. A	Prep. C	Prep. D
1	M	32	20	135	57
2[b]	F	36			48
3	M	24	21		
			84	230	
4	M	25	78	230	114

[a] In minutes. [b] This subject received 75 mg.; all others received 100-mg. doses.

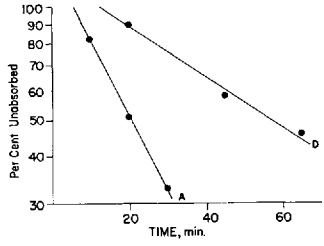

Fig. 2.—Apparent first-order absorption of warfarin from preparations A (subject *3*) and D (subject *1*).

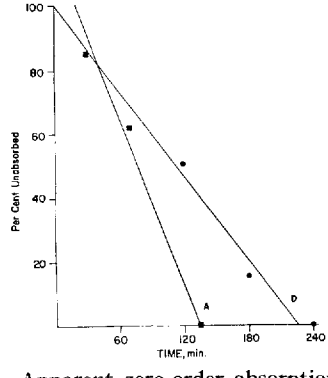

Fig. 3.—Apparent zero-order absorption of warfarin from preparations A (subject *3*) and D (subject *4*).

intra-product differences which overshadowed any product-to-product differences. Based on the dissolution rate experiments, product D was expected to be very slowly and incompletely absorbed. Actually, absorption was quite rapid and complete in each case, as determined from the area under the blood level *versus* time curves and the prothrombin times. The variability of absorption was apparently not due to individual differences between test subjects; subject *3* showed a fourfold variation in absorption half-time on repeated experiments.

Although the variability of absorption made it difficult to obtain blood samples at optimum intervals, the kinetics could be determined in most of the experiments. Preparations A and D, which differed so markedly in the *in vitro* dissolution test, were of particular interest. They were absorbed by both apparent first and zero-order kinetics. Data indicative of apparent first-order absorption are shown in Fig. 2, and examples of apparent zero-order absorption kinetics are depicted in Fig. 3.

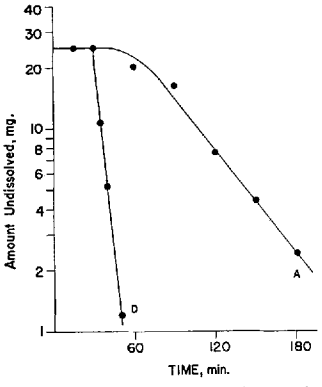

Fig. 4.—Apparent first-order dissolution of warfarin preparations A and D. Conditions: 30 min. dissolution in 0.1 *N* HCl, then dissolution in the medium brought to pH 7.4 by adding tris(hydroxymethyl)aminomethane.

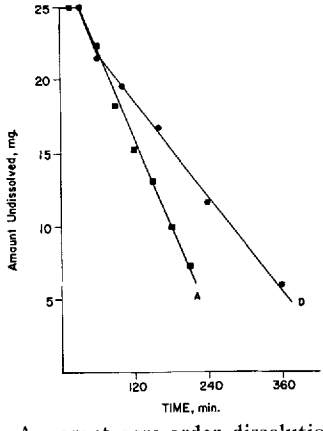

Fig. 5.—Apparent zero-order dissolution of warfarin preparations A and D. Conditions: 30 min. dissolution in 0.01 *N* HCl, then dissolution in the medium brought to pH 7.4 by adding tris(hydroxymethyl)aminomethane.

The marked contrast between the results of the clinical study and those of the dissolution experiments prompted an examination of the conditions of the dissolution procedure and their effect on the dissolution kinetics of warfarin in different tablet preparations. Small changes in stirring rate did not affect significantly the relative dissolution rates of the different preparations. The use of a buffer solution at pH 7.4 instead of water did not result in significant changes. Essentially no dissolution occurred in 0.01 to 0.1 *N* hydrochloric acid solutions because of the extremely low aqueous solubility of warfarin acid. However, tablets A and D disintegrated readily in 0.1 *N* hydrochloric acid but not in water or in 0.01 and 0.05 *N* hydrochloric acid. Based on this observation and the analogy to the passage of drug from the acidic environment of the stomach to the more neutral intestinal fluids, dissolution rates were determined under the following conditions. The tablets were placed in 0.01, 0.05, or 0.1 *N* hydrochloric acid for 30 min., and tris-(hydroxymethyl)aminomethane was then added to increase the pH of the medium to 7.4.

Under these conditions tablets A and D dissolved by apparent first-order kinetics after exposure to 0.1 *N* hydrochloric acid (Fig. 4), and by apparent zero-order kinetics after exposure to 0.05 or 0.01 *N* hydrochloric acid (Fig. 5). While tablets A dissolved about 350 times more rapidly than tablets D in water (Fig. 1), tablets D dissolved about 14 times faster than tablets A in pH 7.4 buffer after exposure to 0.1 *N* hydrochloric acid (Fig. 4).

The results of the dissolution experiments with hydrochloric acid solutions as the initial medium correlate with the clinical studies, and serve to explain the surprisingly rapid absorption of the warfarin acid tablets. The dissolution data, together with physiologic considerations, may also explain the qualitative and quantitative variability observed in the absorption study. Warfarin is almost insoluble in acidic gastric fluids and is therefore apparently not absorbed from the stomach. This has been demonstrated already in previously reported experiments (9) and may account for the lag times for absorption. For example, the longer time required by subject *1* to absorb warfarin from solution than from tablets A probably resulted from fortuitously rapid gastric emptying of the tablets in the latter instance. The variation in the type of absorption kinetics observed (*i.e.*, zero-order or first-order) can result from variations in the pH of gastric fluids within the physiologic range (13). Thus, absorption of warfarin from tablets A and D is determined in part by gastric emptying rate and gastric pH. Absorption from tablets C is probably influenced mainly by gastric emptying, since dissolution was not affected markedly by variations in the concentration of hydrochloric acid in the *in vitro* experiments.

The results of this study show the pronounced effect of solvent composition on dissolution kinetics of drugs in certain tablet formulations. The study illustrates also the difficulty of obtaining correlations with *in vitro* dissolution rates when absorption is influenced markedly by gastric emptying rate. Similar results were obtained recently with enteric-coated tablets (14). Because of the long half-life of warfarin (10), the blood levels of this drug are relatively insensitive to variations in absorption rate, unless absorption is so slow that the drug is absorbed incompletely. With more rapidly eliminated drugs, wide variations in absorption rates could limit seriously their usefulness for oral administration, even if complete absorption occurs in each case. The need for pretreatment with acid in order to obtain rapid dissolution, observed with tablets A and D, suggests that these tablets have a matrix which dissolves or disintegrates readily only in acid. It would be desirable that, in most instances, conventional compressed tablets be so formulated that release of the active ingredient is not a sensitive function of pH in the physiologic range.

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