Table II—Biological Half-Life of Various Penicillins in Male and Female Beagle Dogs after Intravenous Administration

Sex	Half-Life, min.		
	Nafcillin[a]	Dicloxacillin[a]	Ampicillin
Male	10 (9–11)[b]	16 (12–20)[b]	35 (29–39)[b]
Female	10 (9–11)[b]	18 (15–22)[b]	34 (28–38)[b]

[a] Administered as the sodium salt of the monohydrate. [b] Range of half-life observed.

In any event, the sex of the test animal should be considered in studies concerned with *in vivo* effects of monobasic penicillins in which dogs, particularly beagles, are used. The effect of the animal's sex on the biological utilization of such compounds in other species and in human subjects is not known at this time. Studies in humans are of interest since differences of the magnitude observed in the animal studies may be clinically significant.

SUMMARY

The biological utilization, as demonstrated by blood serum levels and area under the serum level–time curves, for a series of penicillin compounds in male and female beagle dogs has been determined. Results obtained indicate that the female of this species attained a higher mean peak serum level and a greater area under the blood serum level–time curve than did the male after oral administration of the monobasic penicillins studied: dicloxacillin, nafcillin, and penicillin G. The amphoteric penicillins employed, ampicillin and Wy-4508, showed no significant difference in blood serum levels or curve areas after oral administration to male or female beagle dogs. There was no significant difference between the sexes in the elimination rate (half-life) of the monobasic penicillins, sodium dicloxacillin monohydrate and sodium nafcillin monohydrate, or of the amphoteric penicillin, ampicillin, after intravenous administration, which rules out this factor as an explanation for the difference in serum levels noted. A possible reason for the sex difference in serum levels in beagle dogs may be due to a difference in acidity of the gastrointestinal tract and/or to a difference in the gut wall metabolism of the monobasic penicillins in the male and female of this species.

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ACKNOWLEDGMENTS AND ADDRESSES

Received February 11, 1970, from the *Pharmacy Research and Development Division, Wyeth Laboratories, Radnor, PA 19087*

Accepted for publication April 17, 1970.

The author thanks G. Owen for administering the drugs and obtaining blood serum samples, G. Warren and S. Rosenman for the microbiological analysis, and R. Levi for preparation of the various experimental formulations.

Presented to Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, Montreal meeting, May 1969.

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Pharmacokinetic Analysis of Potentiating Effect of Phenylbutazone on Anticoagulant Action of Warfarin in Man

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Abstract □ Warfarin is eliminated more rapidly but its anticoagulant effect is increased by concomitant administration of phenylbutazone. Pharmacokinetic analysis by recently developed techniques shows that the prewarfarin synthesis rate and the normal degradation of prothrombin complex activity are not affected by phenylbutazone, but that this drug has a pronounced effect on the relationship between synthesis rate of prothrombin complex activity and plasma–warfarin concentration. These observations are consistent with the assumption that phenylbutazone competitively displaces warfarin from nonspecific binding sites in the plasma and tissues (particularly the liver) and thereby increases the interaction of the anticoagulant with its pharmacologic receptor and metabolizing enzyme system.

Keyphrases □ Warfarin activity—phenylbutazone effect □ Phenylbutazone effect, warfarin activity—pharmacokinetics □ Pharmacokinetics—phenylbutazone potentiation, warfarin activity □ Biologic half-life, warfarin—phenylbutazone effect

Drug interactions are usually of three types. A drug may increase or decrease the elimination rate constant of another by stimulating or inhibiting drug metabolizing enzymes, by changing urine pH or the flow rate of urine or bile, and/or by affecting the distribution of the other drug in the body. Another type of drug interaction involves the potentiation or inhibition of the pharmacologic effect of one drug by another without measurably affecting its kinetics of elimination. The third type of drug interaction is one where the gastrointestinal absorption of one drug is increased or decreased by the other. The coumarin anticoagulants exemplify all three of these effects. Induction of microsomal enzymes by heptabarbital increases the rate of elimination of warfarin and bishydroxycoumarin without affecting the relationship between anticoagulant effect and plasma–coumarin concentration in man (1, 2). Heptabarbital apparently also decreases the gastrointestinal absorption of bishydroxycoumarin (3) by mechanisms which are still being studied.

A particularly interesting interaction is that between warfarin and phenylbutazone. The former is more rapidly eliminated in the presence of phenylbutazone, but its anticoagulant effect in man is increased (4).

Table I—Effect of Phenylbutazone (PB) on the Elimination of Warfarin[a]

Subject[b]	Age, yr.	Weight, kg.	Date of Experiment Control	Date of Experiment With PB	Biologic Half-Life,[c] hr. Control	Biologic Half-Life,[c] hr. With PB	Extrapolated Initial Plasma-Warfarin Concn.,[c,d] mg./l. Control	Extrapolated Initial Plasma-Warfarin Concn.,[c,d] mg./l. With PB
N-22	22	58	8-7-67	5-21-67	55	37	14.0	17.0
N-27	22	78	6-26-67	7-17-67	59	42	15.7	17.3
N-30	23	70	12-13-67	11-27-67	60	32	16.1	17.0
N-31	25	84	12-17-67	11-27-67	79	51	13.2	13.9
N-32	21	62	8-7-67	8-25-67	59	42	13.6	12.8
N-33	21	85	12-17-67	11-27-67	72	35	13.6	17.2
Mean					64	40	14.3	15.9
SD					9	7	1.2	2.0

[a] Sodium warfarin, 1.5 mg./kg. body weight, alone and on the 4th day of phenylbutazone administration, 200 mg. three times a day. [b] All males. [c] Based on least-squares regression analysis of log C_p values >1 mg./l. [d] Listed only to facilitate description of the data. These values are a complex function of the amount absorbed and of the kinetics of absorption, distribution, and elimination.

This is unusual since more rapid elimination of a drug generally results in a decrease in its total pharmacologic effect (unless the drug is activated by metabolism). For example, the total anticoagulant effect of warfarin is decreased by enzyme inducers such as heptabarbital (1). Recently developed pharmacokinetic techniques (5) now permit a more detailed assessment of the mechanism of potentiation of warfarin by phenylbutazone.

EXPERIMENTAL

A single dose of sodium warfarin, 1.5 mg./kg. body weight, was administered orally to six normal subjects. Plasma-warfarin concentrations and prothrombin complex activity were determined daily for 5 days or more. The same experiment was carried out also during phenylbutazone (PB) administration (200 mg. orally three times a day for at least 8 days). Warfarin was administered on the 4th day of PB administration. Additional details of the study are described elsewhere (4, 6).

THEORETICAL

The theory and methodology for relating the anticoagulant effect of warfarin to its concentration in the plasma have been described in detail in a previous report (5) and will only be summarized here. Coumarin anticoagulants interfere with the synthesis of vitamin K-dependent blood-clotting factors (Factors II, VII, IX, and X) but do not affect their degradation. There is no direct relationship between prothrombin complex activity (P) and warfarin concentration in the plasma (C_p), because the former represents the net effect of the synthesis of various clotting factors and their degradation. Thus,

$$R_{net} = R_{syn.} - R_{deg.} \qquad \text{(Eq. 1)}$$

where R_{net} is the net rate of change in P, $R_{syn.}$ is the rate of synthesis, and $R_{deg.}$ is the rate of degradation of P. Since $R_{deg.} = k_d P$,

$$R_{net} = R_{syn.} - k_d P \quad \text{or} \quad R_{syn.} = R_{net} + k_d P \qquad \text{(Eq. 2)}$$

where k_d is the apparent first-order degradation rate constant for P. Prior to the administration of a coumarin anticoagulant,

$$R_{syn.}^0 = k_d P^0 \qquad \text{(Eq. 3)}$$

In the clinical range of C_p and $R_{syn.}$, there is an essentially linear relationship between $R_{syn.}$ and log C_p which, as has been shown previously (5), is described by the relationship

$$R_{syn.} = R_{syn.}^0 + m \log C_{p\,min.} - m \log C_p \qquad \text{(Eq. 4)}$$

where $C_{p\,min.}$ is the hypothetical minimum effective C_p. A plot of $R_{syn.}$ *versus* log C_p has a slope of $-m$ and extrapolates to $C_{p\,max.}$ at $R_{syn.} = 0$. P and C_p are determined experimentally as a function of time. Administration of a synthesis-blocking dose of warfarin (such that $R_{syn.} = 0$) yields k_d. The change of P with time determines R_{net}. $R_{syn.}$ can then be calculated by means of Eq. 2. Least-squares regression analysis of $R_{syn.}$ as a function of log C_p yields m and $C_{p\,max.}$

RESULTS

PB significantly decreased ($p < 0.01$ by paired t test) the biologic half-life of warfarin from an average of 64 hr. to an average of 40 hr. (Table I). The extrapolated initial C_p was higher during PB administration in five of the six subjects, but the difference was not statistically significant. P^0 was 100% of normal in all subjects in the control period and during PB administration. Also, PB had no effect on k_d and, therefore, did not change $R_{syn.}^0$ (Table II).

The relationship between $R_{syn.}$ and log C_p was profoundly altered by PB. In three of the six subjects the relationship remained approximately linear, but the slope of the regression line ($-m$) decreased appreciably (*e.g.*, Fig. 1). In the other three subjects, data obtained during PB administration show slight curvature (*e.g.*, Fig. 2). Despite

Table II—Effect of Phenylbutazone (PB) on Synthesis Rate ($R_{syn.}^0$) and Degradation Rate Constant (k_d) for Prothrombin Complex Activity

Subject	$R_{syn.}^0$, % of Normal/Day Control	$R_{syn.}^0$, % of Normal/Day With PB	k_d, day^{-1} Control	k_d, day^{-1} With PB
N-22	101	111	1.01	1.11
N-27	95	95	0.95	0.95
N-30	107	107	1.07	1.33[a]
N-31	95	92	0.95	0.92
N-32	92	92	0.92	0.92
N-33	96	96	0.96	0.96
Mean	98	99	0.98(0.98)[b]	1.03(1.01)[b]
SD	5	8	0.05	0.16

[a] Questionable value based on two data points. Control k_d value was used for all calculations. [b] Harmonic mean.

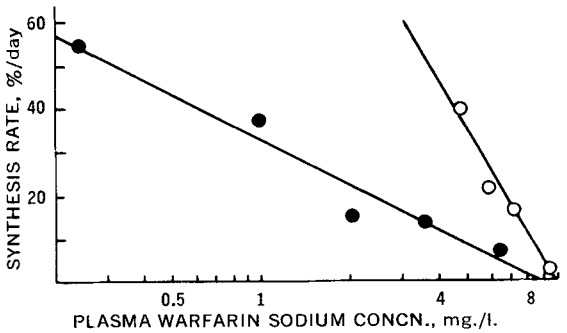

Figure 1—*Effect of phenylbutazone on the relationship between synthesis rate of prothrombin complex activity* ($R_{syn.}$) *and plasma warfarin concentration* (C_p) *in Subject N-33. Key:* ○, *control; and* ●, *with phenylbutazone.*

Table III—Effect of Phenylbutazone (PB) on the Relationship between Prothrombin Complex Activity Synthesis Rate and Plasma Warfarin Concentration

Subject	m, % of Normal/Day		Correlation Coefficient		$C_{p\ max.}$, mg./l.	
	Control	With PB	Control	With PB	Control	With PB
N-22	145	35	0.99	0.96	12.8	11.0
N-27	181	53	0.98	0.95	12.4	8.1
N-30	128	46	0.96	0.92	12.0	8.8
N-31	132	16	0.99	0.85	10.4	20.0
N-32	72	56	0.92	0.96	12.2	6.1
N-33	119	35	0.98	0.98	9.8	8.6
Mean	130	40			11.6	10.4
SD	36	15			1.2	5.0

this, and merely for facilitating a summary of the results, regression lines were fitted also to these data by the method of least squares. This summary is presented in Table III. There was a highly significant ($p < 0.01$) decrease in m during PB administration but no statistically significant change in $C_{p\ max.}$ (by paired t test), although five out of six values were lower during PB administration. Figure 3 shows the results obtained in Subject N-31 in the present study, as well as the results of three additional experiments with warfarin alone. C_p values in the additional experiments were determined spectrophotometrically, while all C_p values in the present study were determined by fluorometry.

DISCUSSION

The biologic half-life of warfarin was significantly decreased during PB administration. PB is a known inducer of drug-metabolizing enzymes (7) but, due to its pronounced affinity to plasma proteins (8), it also displaces warfarin from protein-binding sites (6). There is evidence that decreased plasma protein binding increases the elimination rate constant of coumarin anticoagulants by increasing their concentration at biotransformation sites in the liver (9, 10). On the other hand, PB and bishydroxycoumarin (and, therefore, presumably also warfarin) may be metabolized by the same enzyme system (11); therefore, there may occur mutual inhibition of biotransformation. Thus the observed effect of PB on the elimination of warfarin may be the net result of more than one type of effect, and its direction and magnitude may depend on the dose and duration of PB administration.

The lack of effect of PB on P^0, k_d, and, therefore, on $R_{syn.}{}^0$ shows that PB has no apparent effect on the blood-clotting system. On the other hand, the relationship between $R_{syn.}$ and C_p is considerably changed by PB. Unfortunately, the fluorometric assay for warfarin [which had to be used in this study because PB interferes with the spectrophotometric assay (6)] is not totally specific for unchanged warfarin and is affected also by one or more of its hydroxylated metabolites (12). Particularly the lower C_p values (*i.e.*, the values obtained late in the experiment) are, therefore, somewhat higher than those obtained by spectrophotometry. Consequently, as is exemplified in Fig. 3, the regression lines are steeper (and m values larger) than when C_p is determined spectrophotometrically. However, this has little effect on the *relative* relationships between $R_{syn.}$ and C_p with and without PB.

The effect of PB is unlike that produced by the enzyme inducer heptabarbital (1) and differs also from the pattern observed in a subject who is genetically resistant to warfarin (13). Heptabarbital stimulates the biotransformation of warfarin and, therefore, decreases C_p; but the anticoagulant effect at a given C_p is the same during control periods and during heptabarbital administration, *i.e.*, the "concentration–response" relationship is not altered (1). In the resistant subject, the slope of the $R_{syn.}$ *versus* log C_p plot is normal, but the regression line is displaced laterally to a higher $C_{p\ max.}$ value (13).

PB affects mainly the slope ($-m$) of the $R_{syn.}$–log C_p plot. Qualitatively, this suggests that PB competitively displaces warfarin from nonspecific protein-binding sites in the plasma and tissues[1] and thereby affords more extensive interaction of the anticoagulant with its pharmacologic receptor in the liver. The more rapid elimination of warfarin during PB administration is consistent with this reasoning, since warfarin is eliminated by biotransformation in the liver and the elimination rate of the anticoagulant is a function of its concentration in the liver. The warfarin–PB interaction is an interesting example of the simultaneous occurrence of two separate effects, enhanced elimination (which ordinarily would decrease the total pharmacologic effect) and potentiation of the pharmacologic

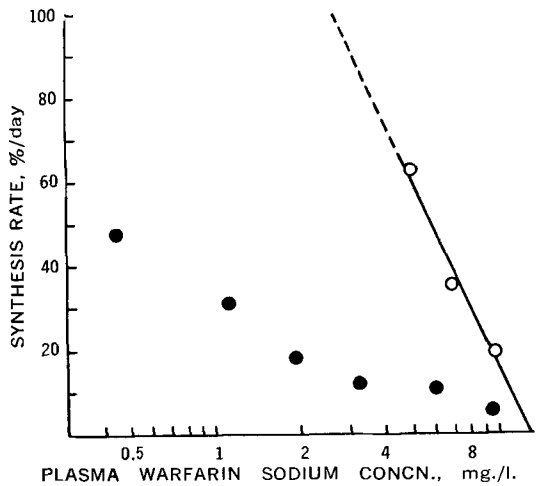

Figure 2—*Effect of phenylbutazone on the relationship between* $R_{syn.}$ *and* C_p *in Subject N-22. Key:* ○, *control; and* ●, *with phenylbutazone.*

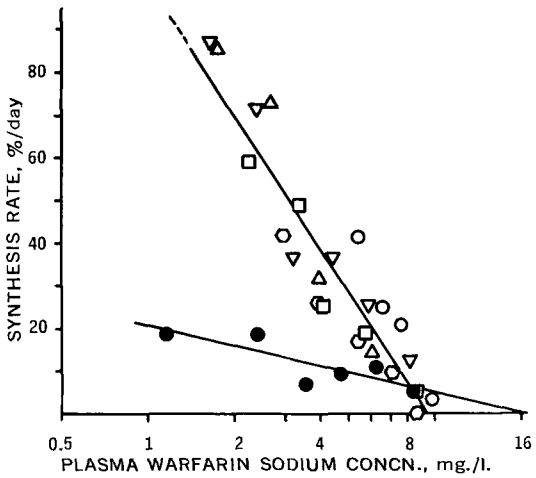

Figure 3—*Effect of phenylbutazone on the relationship between* $R_{syn.}$ *and* C_p *in Subject N-31. Key:* ○, *control; and* ●, *with phenylbutazone. Shown also are data for four other control experiments in which* C_p *was determined spectrophotometrically. The regression line for the control results was fitted to all the experimental data.*

[1] Note, for example, the qualitative similarity between the effect of a constant concentration of displacing agent on the protein binding of a drug at various concentrations (Fig. 3 in *Reference 14*) and the effect of a presumably relatively constant body level of PB on the activity of warfarin as found in the present study.

action of warfarin. Both of these effects seem to be due primarily to a change in the distribution of warfarin in the body.

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ACKNOWLEDGMENTS AND ADDRESSES

Received October 28, 1969, from the *Department of Medicine, Santa Clara Valley Medical Center and the Institute for Medical Research of Santa Clara County, and the †Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Buffalo, NY 14214

Accepted for publication April 30, 1970.

Supported in part by Grant HE 8058 from the U. S. Public Health Service.

The technical assistance of Geraldine M. Keech is gratefully acknowledged.

Diffuse Reflectance Studies of Solid–Solid Interactions

JOHN L. LACH and LYLE D. BIGHLEY*

Abstract □ Tetracycline and its derivatives, bishydroxycoumarin, and methantheline bromide have been studied by diffuse reflectance spectroscopy for possible solid–solid interactions with various metallic adjuvants. Examination of the spectra of some of these drug–adjuvant systems show rather small spectral changes, while other spectra show large bathochromic and hyperchromic changes, new band formation, and visual color changes which are indicative of charge–transfer interactions. Although these interactions vary in variety and intensity, they may significantly alter the availability and activity of the medicinal agent in pharmaceutical dosage forms.

Keyphrases □ Solid–solid interaction—determination □ Diffuse reflectance spectroscopy—solid–solid interaction analysis □ Tetracyclines, methantheline bromide, bishydroxycoumarin—metal-ion interactions □ Metal ions–tetracyclines, –methantheline bromide, –bishydroxycoumarin—interactions

Many articles in the pharmaceutical and medical literature deal with the problem of physiologically inactive tablet and capsule formulations. Lach and Bornstein (1), by the use of diffuse reflectance spectroscopy (DRS), have postulated that the apparent inactivity may be due in part to adsorption of the active principle onto an inert adjuvant. They have shown that solid–solid interactions do indeed exist between various chemical entities and a wide variety of adjuvants commonly found in pharmaceutical dosage forms. Although some of the interactions may be of the weak variety, they may, nevertheless, be sufficient to alter the absorption and availability of the medicinal agent.

The study of solid–solid interactions is of interest because of the effects that adjuvants may exhibit when incorporated into pharmaceutical dosage forms. These effects may include: (*a*) changes in the nature and intensity of biological activity caused by complexation with the active ingredient (2); (*b*) modifications of the physical state, particle size, and/or surface area of the drug available to the absorption sites (3); or (*c*) changes in the stability of the active principle (4–7).

The purposes of this study were to continue the investigation of solid–solid interactions of a number of drugs and to gain a better insight into the nature of these interactions.

EXPERIMENTAL

Reagents—Oxytetracycline hydrochlorde,[1] tetracycline hydrochloride,[2] chlortetracycline hydrochloride,[2] demethylchlortetracycline hydrochloride,[2] methantheline bromide,[3] bishydroxycoumarin,[4] magnesium trisilicate, ferric phosphate, aluminum hydroxide, tribasic calcium phosphate, and talc were used.

Apparatus—The following were used: Beckman model DU spectrophotometer with a diffuse reflectance attachment (1), constant-temperature water bath with rotating spindles, 150-ml. amber vials with caps, Parafilm,[5] and a glass desiccator with anhydrous calcium sulfate.[6]

Procedure—*Preparation of the Tetracyclines*—Thirty milligrams of active ingredient (tetracycline HCl, oxytetracycline HCl, chlortetracycline HCl, and demethylchlortetracycline HCl) was weighed for every 2 g. of adsorbent (pharmaceutical adjuvant) used. The powders were placed in 150-ml. amber vials, and 25 ml. of distilled water was added as the dispersion medium. The vial was then covered with Parafilm and capped. Equilibration was allowed to proceed for 2 hr. at 30 ± 0.5° to effect interaction. After equilibration, the suspension was filtered under vacuum and the powder dried

[1] Pfizer and Co.
[2] Lederle and Co.
[3] Searle and Co.
[4] K and K Laboratories.
[5] Marathon Co.
[6] Drierite, Hammond Co.