Effect of warfarin on the metabolism of phylloquinone (vitamin K₁): dose–response relationships in man

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Summary

1. The dose–response relationship between the oral anticoagulant, warfarin, and its effect on the metabolism of phylloquinone (vitamin K₁) has been examined in normal male volunteer subjects.

2. In each study the subject received a single, oral dose of warfarin and, 2 h later, an intravenous injection of [1',2'-3H₂]phylloquinone. Changes in the metabolism of phylloquinone were assessed by the fractionation and chromatographic separation of labelled phylloquinone and its metabolites in plasma and urine samples.

3. Increasing doses of warfarin did not affect the rate of disappearance of injected phylloquinone from the plasma but caused the accumulation of increasing amounts of the metabolite, phylloquinone epoxide.

4. Increasing doses of warfarin were found to decrease the proportion of labelled conjugates excreted in the urine as glucuronides and to block progressively the excretion of the normal aglycones of phylloquinone. At the same time there was a progressive increase in the excretion of at least three abnormal aglycones of phylloquinone.

5. The doses or plasma concentrations of warfarin were related to the increase in phylloquinone epoxide in the plasma and to the decrease in the proportion of normal aglycones of phylloquinone in urine by typical log dose–response curves, which were linear over the therapeutic range.

6. The nature of the metabolites detected suggested that the dose–response curves reflected the progressive inhibition by warfarin of the enzyme, phylloquinone epoxide reductase.

7. The results are consistent with the hypothesis that the pharmacological action of oral anticoagulants is closely linked to their ability to inhibit the cyclic interconversion of vitamin K and vitamin K epoxide.

Key words: dose–response relationship (drug), phylloquinone reductase, phytonadione, vitamin K, warfarin.

Introduction

Over the last few years evidence has grown that the ability of coumarin and indanedione anticoagulants such as warfarin and phenindione to interfere with the synthesis of the vitamin K-dependent clotting factors is linked to the inhibition of a specific enzymic step in the metabolism of vitamin K itself. During the normal synthesis of factors II (prothrombin), VII, IX and X it has been postulated that vitamin K shuttles between its native biologically active form and an inactive, 2,3-epoxide metabolite (Bell & Matschiner, 1972). Since the work concerning this aspect of vitamin K metabolism has utilized phylloquinone (vitamin K₁), the cyclic interconversion between the vitamin and its 2,3-epoxide metabolite has been termed the vitamin K₁–epoxide cycle.
This cycle depends upon at least two enzymes, which have been partially characterized as phylloquinone epoxidase (Willingham & Matschiner, 1974) and phylloquinone epoxide reductase (Matschiner, Zimmerman & Bell, 1974; Zimmerman & Matschiner, 1974). Matschiner et al. (1974) have shown that warfarin inhibits phylloquinone epoxide reductase in vitro, thus explaining their earlier findings that in vivo large amounts of phylloquinone 2,3-epoxide accumulated in the livers of warfarin-treated rats (Matschiner, Bell, Amelotti & Knauer, 1970). They have further proposed that the vitamin K-dependent step in the synthesis of the clotting factors is linked to the epoxidation of the vitamin (Willingham & Matschiner, 1974) and that the role of the reductase is to regenerate vitamin K (Zimmerman & Matschiner, 1974).

In clinical practice, where oral anticoagulants are widely used for the prophylaxis and treatment of thrombo-embolic disease, all the current drugs are structurally related to either coumarin or indanedione. Representative drugs from each of these two groups have so far all been found to cause the accumulation of phylloquinone 2,3-epoxide in the liver of rats by inhibiting reduction of the epoxide to the vitamin (Ren, Laliberte & Bell, 1974; Sadowski & Suttie, 1974). In humans we have previously reported that phylloquinone 2,3-epoxide also accumulates in the plasma of warfarin-anticoagulated subjects (Shearer, McBurney & Barkhan, 1973). These results suggest that all current clinical oral anticoagulants are inhibitors of the vitamin K₁-epoxide cycle and that further insight into their mode of action in man might be gained from a study of their effect on the metabolism of phylloquinone.

Apart from measurements of the accumulation of phylloquinone 2,3-epoxide in the plasma, the isolation and characterization of the normal metabolites of phylloquinone from human urine (Shearer & Barkhan, 1973; Shearer, McBurney & Barkhan, 1974) provides a further technique to study the effect of warfarin on the metabolism of phylloquinone. We have shown that warfarin alters the pattern of conjugates in urine and blocks the excretion of the normal aglycones of phylloquinone in urine (Shearer et al., 1974).

Whereas in our previous studies subjects were maintained on therapeutic doses of warfarin for several days before the vitamin study, we have now measured the effect of different single doses of warfarin on the metabolism of a tracer dose of [1',2'-³H₂]phylloquinone. It was thought that changes in the metabolite pattern of phylloquinone in the plasma and urine might mirror the effect of each dose of warfarin on the vitamin K₁-epoxide cycle in the liver.

**Materials and methods**

**Materials**

[1',2'-³H₂]Phylloquinone, specific radioactivity 98 Ci/mol (217 µCi/mg) (a gift from Hoffmann–La Roche and Co., Basle, Switzerland), was received as a micellar solution in sealed sterile ampoules containing 10 µCi (45 µg) per 0.5 ml of solvent (polyethylene glycol ester). Phylloquinone 2,3-epoxide was synthesized from phylloquinone by the method of Tishler, Fieser & Wendler (1940).

Warfarin [3-(a-acetonylbenzyl)-4-hydroxycoumarin] was obtained from BDH Ltd (Poole, Dorset, U.K.) as tablets of Marevan containing 1 mg, 5 mg or 10 mg of racemic sodium warfarin. The R(+) and S(−) enantiomers of acidic warfarin were a gift from Ward Blenkinsop Pharmaceuticals (Wembley, Middlesex, U.K.) and had optical purities of 92.5% and 97.3% respectively.

**Subjects**

Fourteen studies were conducted in eight normal males aged 20–32 years and weighing 51–78 kg. Approval for the administration of radioactive phylloquinone was obtained from the Isotope Advisory Panel of the Medical Research Council. Permission for the study was obtained from the Committee on Ethical Practice of Guy's Hospital and Medical School, and all subjects gave their informed consent.

**Plan of study**

For each warfarin study, the subject fasted overnight and ingested the dose of warfarin at about 08.00 hours. Exactly 2 h later 45 µg (10 µCi) of [1',2'-³H₂]phylloquinone was injected intravenously into a forearm vein. The subjects were allowed liquids at any
time and 2 h after the injection of phylloquinone they were allowed to continue their normal diet. The doses of warfarin administered in each study ranged from 1 to 70 mg. Further details of subjects and the warfarin dosage scheme are given in Clinical Science and Molecular Medicine Tables 77/04 deposited with the Librarian, The Royal Society of Medicine (1 Wimpole Street, London W1M 8AE), from whom copies can be obtained on request.

In a control experiment, one subject who had fasted overnight was injected with 45 µg (10 µCi) of [1',2'-3H₂]phylloquinone only.

To investigate the effect of 50 mg loading doses of the separate R and S enantiomers of warfarin, two studies were carried out in the same subject. Each enantiomer was administered in solution as described by Breckenridge & Orme (1973). The effect of a 50 mg loading dose of racemic warfarin on the metabolism of different doses (45 and 1045 µg) of phylloquinone was investigated in two studies in the same subject. For the larger dose of phylloquinone 45 µg (10 µCi) of [1',2'-3H₂]phylloquinone was mixed with 1 mg of unlabelled phylloquinone (Konakion, Roche Products Ltd, Welwyn Garden City, Herts., U.K.) and the total injected 2 h after the oral administration of 50 mg of racemic warfarin. After the injection of [1',2'-3H₂]phylloquinone, venous blood was sampled after 0-5, 1, 2, 3, 4, 6 and 8 h. Urine collections were pooled for the periods 0–8, 8–24 and 24–48 h. For the subjects given 70 mg of racemic or 50 mg of S warfarin precautions were taken to prevent the continuing anticoagulant effect of these large doses by the oral administration of 20 mg of phylloquinone (Konakion) 8 h after the injection of the labelled phylloquinone.

**General procedures**

**Prothrombin times.** These were determined as previously described (Shearer et al., 1973).

**Plasma warfarin concentrations.** These were determined by the method of Lewis, Ilnicki & Carlstrom (1970), which measures the R and S enantiomers equally.

**Radioactivity measurements.** Scintillation counting was performed as previously described (Shearer & Barkhan, 1973).

**Lipid extraction.** Total lipid extracts of plasma samples (1–4 ml) were prepared as previously described (Shearer, Barkhan & Webster, 1970), except that the step in which the aqueous methanol phases were washed with chloroform was omitted.

**Measurement of [1'H]phylloquinone and [3H]-phylloquinone 2,3-epoxide in plasma.** Total lipid extracts of plasma were subjected to reversed-phase thin-layer chromatography as described by Shearer et al. (1973) except that the support was Kieselgel GF₂₅₄ and the developing solvent was acetone/water (88:12, v/v).

**Total radioactivity of urine.** This was measured by direct radioactivity counting of 0.3 ml aliquots as previously described (Shearer et al., 1970).

**Fractionation of ³H-labelled metabolites from urine.** The first 8 h urine collection from each subject was fractionated as follows.

1. To obtain the acidic fraction, urine adjusted to pH 2 was continuously extracted with diethyl ether for 20 h (Shearer & Barkhan, 1973).

2. To obtain the aglycone fraction, ether extracts from (1) above were subjected to either (a) β-glucuronidase hydrolysis as described by Shearer & Barkhan (1973) or (b) solvolysis in ethyl acetate by one of the procedures described by Burstein & Lieberman (1958). For solvolysis, ether extracts of urine were dissolved in water, adjusted to pH 1 with HCl (1 mol/l) and sodium chloride added to a final concentration of 200 g/l. The mixture was extracted once with an equal volume of ethyl acetate, and the upper ethyl acetate phase removed and incubated at 37°C for 24 h. The solvent was removed under a stream of N₂.

3. The aglycones obtained from (2) above were either (a) distributed between chloroform and water or (b) methylated with diazomethane and subjected to adsorption thin-layer chromatography. Details of these procedures have been given previously (Shearer & Barkhan, 1973). For thin-layer chromatography the solvent system was chloroform/diethyl ether (9:1, v/v). After development the distribution of radioactivity in 1 cm bands of adsorbant was determined as previously described (Shearer & Barkhan, 1973).

**Results**

**Plasma measurements**

**Warfarin.** Details of the plasma concentration
of warfarin at various times after the oral administration of single doses of racemic warfarin or its R and S enantiomers are given in the deposited Clinical Science and Molecular Medicine Tables 77/04 referred to above.

Disappearance of [3H]phylloquinone. Over the period 0–8 h the form of the curve of the disappearance of unchanged phylloquinone from the plasma was similar for all subjects and as previously reported (Shearer et al., 1974) could be resolved graphically into two major exponential components.

The $t_{0.5}$ values for these exponential components ranged from 23 to 36 min for the fast component and from 116 to 260 min for the slow component. There was no relationship, however, between these half-lives and the dose of warfarin administered.

[3H]Phylloquinone 2,3-epoxide. The plasma time-course of [3H]phylloquinone 2,3-epoxide over 8 h for the control study and for five representative studies in subjects who were given 1, 3, 5 or 10 mg doses of racemic warfarin or 50 mg of S warfarin are shown in Fig. 1.

After 1 mg (14 $\mu g$/kg body wt.) of racemic warfarin, the plasma concentrations of [3H]-phylloquinone epoxide fell continuously after injection of [1',2'-2H$_2$]phylloquinone and were similar to those found in the control study. In both these studies, the low but significant radioactivity associated with carrier phylloquinone epoxide probably represented trailing from more polar metabolites which travelled above phylloquinone epoxide in the reversed-phase thin-layer-chromatography system.

After 3 mg (39 $\mu g$/kg body wt.) of racemic warfarin, the plasma concentrations of [3H]phylloquinone epoxide were raised and from 1 to 8 h were two to three times greater than the corresponding values for the control study (Fig. 1). Increasing the dose of warfarin caused increased amounts of phylloquinone epoxide to accumulate in the plasma (Fig. 1). For higher doses of warfarin, the time-courses of phylloquinone epoxide in the plasma resembled those previously observed in subjects maintained on therapeutic doses of warfarin (Shearer et al., 1973, 1974). In all subjects peak concentrations of [3H]phylloquinone epoxide in the plasma occurred 2–4 h after the injection of phylloquinone; thereafter the plasma concentration fell exponentially with $t_{0.5}$ values ranging from 3 to 12 h.

If the accumulation of phylloquinone epoxide in the plasma is considered as a measure of the response to a given dose of warfarin, a dose–response curve may be constructed in which the log of the warfarin dose is plotted against the peak plasma concentrations of phylloquinone epoxide. For various doses of racemic warfarin, a typical sigmoidal dose–response curve was obtained (Fig. 2), which was essentially linear for doses between 50 and 300 $\mu g$/kg body wt. This dose range corresponds to single doses of warfarin of about 3–20 mg and approximates to the daily doses required for anticoagulant therapy.

Since for each study the plasma concentrations of warfarin did not vary greatly over the 8 h period during which estimations of [3H]phylloquinone epoxide were made, we also examined the relationship between the mean plasma warfarin concentrations over this period and the peak values of [3H]phylloquinone epoxide. There was a correlation ($P<0.001$) between the log of the plasma warfarin concentration and the peak plasma value of [3H]phylloquinone epoxide, the regression line being given by the equation $y = 306.9 \log x +$
Warfarin and metabolism of phylloquinone 625

![Graph](image)

**Fig. 2.** Relationship between the maximum concentration of \(^{3}H\)phylloquinone epoxide in the plasma and the log of the warfarin dose. ○, Subjects given various doses of racemic warfarin; ● and □, subject given doses of 764 μg/kg of S (●) or R (□) warfarin.

277.6 (r = 0.94, n = 9), where x represents the plasma warfarin concentration.

**Urinary measurements**

Phylloquinone is normally excreted in human urine as two closely related compounds which have been identified as the glucuronide conjugates of 2-methyl-3-(5'-carboxy-3'-methyl-2'-pentenyl)-1,4-naphthaquinone (metabolite I), and 2-methyl-3-(3'-carboxy-3'-methylpropyl)-1,4-naphthaquinone (metabolite II) (Shearer & Barkhan, 1973; Shearer et al., 1974; McBurney, 1975). The excretion of these compounds is greatly reduced in subjects maintained on therapeutic doses of warfarin. Instead abnormal metabolites are excreted which differ from metabolites I and II in the structures of both the conjugate and aglycone moieties (Shearer et al., 1974). One of the objectives of this study was to relate these changes in metabolites in urine to the dose and/or plasma concentration of warfarin.

**Nature of conjugates of phylloquinone in urine.** The total excretion of radioactivity varied between subjects from 3 to 23% for the first 8 h, and from 14 to 36% for the 0–48 h period. The acidic nature of the major fraction of metabolites of phylloquinone in urine was shown by the finding that 61–87% of the radioactivity in the 0–8 h urine collections could be recovered by continuous extraction with diethyl ether at pH 2. There was no relationship, however, between this acidic fraction and the dose of warfarin administered.

Measurements of the distribution of radioactive metabolites between equal volumes of chloroform and water revealed that, after extraction with diethyl ether, about 80% of the radioactivity was still present as water-soluble metabolites, previously attributed to conjugated metabolites (Shearer et al., 1974).

Distribution studies were also used to detect the possible hydrolysis of these conjugates to lipid-soluble aglycones, either enzymically by β-glucuronidase or by the mild physical procedure of solvolysis. With increasing doses of warfarin there tended to be a progressive reduction from 85 to 55% in the fraction of conjugates which was hydrolysed by β-glucuronidase and an increase from 20 to 40% in a more labile conjugate fraction which was hydrolysed by solvolysis.

**Nature of aglycones of phylloquinone in urine.** Molecular differences in the structure of the aglycone moieties of metabolites of phylloquinone in urine were detected by comparing the distribution patterns of radioactivity on thin-layer chromatograms. Representative chromatograms of labelled aglycones obtained by β-glucuronidase hydrolysis are shown in Fig. 3. The chromatogram of the aglycone fraction from the control study (Fig. 3a) revealed only one radioactive peak (peak A₁), which has been previously shown to contain both the normal aglycones of phylloquinone in urine (metabolites I and II) as their methyl esters (Shearer et al., 1974). The effect of doses of 10 mg (197 μg/kg body wt.) and 50 mg (668 μg/kg body wt.) of racemic warfarin on the distribution pattern of radioactivity is shown in Fig. 3(b) and Fig. 3(c). As the dose of warfarin was increased, peak A₁ progressively declined and there was a concomitant increase in the excretion of more polar aglycones, which chromatographed as three distinct peaks (peaks W₁, W₂ and W₃: Fig. 3b and Fig. 3c). Similar distribution patterns of radioactivity were also obtained for aglycone fractions obtained by solvolysis.

For each study, the value for peak A₁, and hence for the normal metabolites I and II, was expressed as a percentage of the value determined for the control study. From duplicate chromatograms in the control study, peak A₁ accounted for 61·6% (range 60·5–62·6%) of the total radioactivity recovered from the thin layers.

The fraction of radioactive aglycones which chromatographed as metabolites I and II was
Effect of warfarin on the metabolism of different doses of phylloquinone

The effect of a 50 mg loading dose of racemic warfarin on the metabolic fate of different doses of phylloquinone was investigated in a subject who in two separate studies was given 45 µg and 1045 µg of phylloquinone respectively. Despite the 20-fold difference in the dose of phylloquinone, the prothrombin times after 24 and 48 h were identical, suggesting that the synthesis of the vitamin K-dependent clotting factors was inhibited to the same extent in each study. Although each dose of phylloquinone was cleared from the plasma at the same rate, the concentrations of phylloquinone epoxide in the plasma were at all times lower for the 1045 µg dose and the peak concentration 22% less than for the 45 µg dose of phylloquinone.

For the metabolites in urine it was found that both the rate and total excretion of radioactive metabolites over the first 72 h was the same for each dose of phylloquinone. The fraction of water-soluble metabolites in the 0–8 h urine collection which was rendered lipid-soluble by β-glucuronidase was also similar. Thin-layer chromatography of the aglycones liberated by β-glucuronidase revealed, however, that the proportion as the normal metabolites I and II was greater for the 1045 µg dose of phylloquinone, when they represented 32.3% of the control value (23.9% for the 45 µg dose).

Effect of R and S enantiomers of warfarin on phylloquinone metabolism

In one subject two studies compared the effect of 50 mg doses of the separate R and S
enantiomers of warfarin on the metabolism of a 45 \( \mu g \) dose of phylloquinone. Although the time-courses and peak concentrations of \([^3H]\)-phylloquinone epoxide over 8 h were very similar for each enantiomer, the accumulation of the epoxide metabolite in the plasma was greater than that for equivalent doses of racemic warfarin (Fig. 2). Measurements of the urinary excretion of phylloquinone showed that there were no differences between the effect of each enantiomer on either the total urinary excretion of radioactivity or on the fraction of labelled conjugates which were hydrolysed by \( \beta \)-glucuronidase. Thin-layer chromatography of the aglycones revealed, however, that \( S \) warfarin was more effective in blocking the urinary excretion of metabolites I and II, the proportion of aglycones as metabolites I and II being reduced to 14.6% of the control value by \( S \) warfarin, compared with 26% by \( R \) warfarin (Fig. 4).

**Relationship between plasma \([^3H]\)phylloquinone epoxide and \(^3H\)-labelled metabolites I and II in urine**

There was an inverse relationship between the peak concentration of phylloquinone epoxide in the plasma and the proportion of \(^3H\)-labelled aglycones in urine as metabolites I and II, the correlation coefficient being highly significant \((P<0.001)\). The regression line was given by the equation \( y = 763.4 - 7.8x \) \((r = -0.93, n = 12)\), where \( x \) represents metabolites I and II.

**Discussion**

**Absorption of warfarin**

It was assumed in the experimental design of these studies that each dose of warfarin would be rapidly absorbed, as shown by Breckenridge & Orme (1973), and completely absorbed, as shown by O'Reilly, Aggeler & Leong (1963). In our studies the plasma concentrations after each dose of warfarin (ranging from 14 to 1000 \( \mu g/kg \)) did not vary significantly over the 8 h period of study. This suggests that the 2 h period between the ingestion of warfarin and the injection of phylloquinone was sufficient to allow for complete absorption of each dose of warfarin.
twofold. First, the increase in the excretion of the abnormal metabolites \(W_1, W_2\) and \(W_3\) was paralleled by a corresponding reduction of the normal metabolites I and II (Fig. 3), and this change in the excretion pattern was correlated with the concentration of phylloquinone epoxide in the plasma. Secondly, several abnormal metabolites of phylloquinone with ultraviolet-absorption spectra very similar to authentic phylloquinone epoxide have been isolated from the urine of warfarin-treated subjects (McBurney, 1975).

The effect of warfarin in reducing the proportion of labelled conjugates that could be hydrolysed by \(\beta\)-glucuronidase has been previously observed in subjects who had been maintained for several days on therapeutic doses of warfarin (Shearer et al., 1974; McBurney, 1975). Evidence has been obtained that this decreased hydrolysis by \(\beta\)-glucuronidase represents a real reduction in the excretion of metabolites of phylloquinone as glucuronides rather than to the presence of an inhibitor of \(\beta\)-glucuronidase in the urine of warfarin-treated subjects (McBurney, 1975). Although no explanation can be offered for this effect, a similar decrease in the glucuronide fraction has been found for the urinary excretion of several steroid hormones after the administration of certain drugs (Bradlow, Zumoff, Fukushima, Hellman, Bickers, Alvares & Kappas, 1973).

**Relationship between inhibition of phylloquinone epoxide reductase and inhibition of prothrombin synthesis**

Although warfarin has been shown to inhibit phylloquinone epoxide reductase in rats both *in vivo* (Matschiner et al., 1970) and *in vitro* (Matschiner et al., 1974), a direct and unequivocal correlation between the degree of this inhibition and the inhibition of prothrombin synthesis has not yet been established. When different coumarin anticoagulants were administered to rats, Sadowski & Suttie (1974) concluded that the hepatic accumulation of phylloquinone epoxide found for each drug did not correlate with their relative effectiveness as anticoagulants. On the other hand, Bell, Caldwell & Holm (1976) did find that the degree of inhibition of prothrombin synthesis by two different coumarin anticoagulants (warfarin and coumatetralyl) was correlated with their inhibition of phylloquinone epoxide reductase, as measured by the ability of rats treated with various doses of these drugs to convert an injected tracer dose of \([3H]\)phylloquinone epoxide into phylloquinone.

The establishment of a correlation is rendered difficult in man by the fact that the degree of inhibition of prothrombin synthesis can only be estimated indirectly from measurements of the plasma levels of factors II, VII, IX and X ('prothrombin complex' activity). Since the
plasma activity of ‘prothrombin complex’ reflects the net effect of both the rate of synthesis and degradation of the vitamin K-dependent clotting factors, the time of the maximum hypoprothrombinaemic response to oral anticoagulants is delayed when compared with the time of the peak concentrations of these drugs in the blood.

In an attempt to relate the anticoagulant effect of warfarin to its concentration in the plasma, Nagashima, O’Reilly & Levy (1969) derived a mathematical expression which defined the pharmacological effect of oral anticoagulants in terms of their effect on the rate of synthesis of ‘prothrombin complex’ activity. They further showed experimentally that when expressed in terms of the rate of synthesis of ‘prothrombin complex’ activity the pharmacological effect of warfarin followed the kinetics expected for a drug–receptor interaction, in which the response is linearly related to the log of the plasma concentration of warfarin.

In our study similar linear relationships were obtained between the log of the dose or plasma concentration of warfarin and specific changes in the metabolism of phylloquinone, as judged by both the accumulation of phylloquinone epoxide in the plasma, and also differences in the molecular nature of the aglycones of phylloquinone excreted in the urine. Our data therefore support the view that there is a close link between the pharmacological action of warfarin and the inhibition of vitamin K metabolism, and that the site of this inhibition is the enzyme phylloquinone epoxide reductase.

**Metabolism of different doses of phylloquinone**

Owing to the lack of quantitative data about vitamin K in the nutrition of man, it is not known how the 45 μg dose of phylloquinone used in our studies compares with the daily intake or body stores of vitamin K. Although Duello & Matschiner (1972) estimated that the concentration of vitamin K was equivalent to only 0-06 μg of phylloquinone/g (wet weight) of liver (suggesting that total human liver reserves of vitamin K are equivalent to about 50–100 μg of phylloquinone), further studies are necessary to establish the variation of vitamin K reserves between individuals.

In subjects maintained on therapeutic doses of warfarin, the time-courses for the accumulation of phylloquinone epoxide in the plasma were similar for both 1 mg and 45 μg doses of phylloquinone (Shearer et al., 1974). In the two studies reported here, the anticoagulant response to 50 mg of warfarin was the same for 45 and 1045 μg doses of phylloquinone. However, the decreased concentrations of phylloquinone epoxide in the plasma after the 1045 μg dose, together with the increased excretion of metabolites I and II in the urine, suggest that the proportion of this dose which was converted into and trapped as the epoxide was slightly less than for the 45 μg dose of phylloquinone. Since these differences in metabolism were small compared with the difference in dose, it is concluded that even quite large variations in the liver reserves of vitamin K would not greatly influence the metabolism of our 45 μg test dose of phylloquinone.

**Effect of R and S enantiomers of warfarin on phylloquinone metabolism**

The R(+) and S(−) enantiomers of which commercial warfarin is an equal mixture have been found to differ both in their rate and pathway of metabolism, and also in their intrinsic potency as anticoagulants. In man S warfarin is three to four times more effective as an inhibitor of prothrombin synthesis than the R enantiomer (Breckenridge, Orme, Wesseling, Lewis & Gibbons, 1974; O’Reilly, 1974).

In a preliminary experiment we found that S warfarin was about twice as effective as R warfarin in blocking the urinary excretion of metabolites I and II. On the other hand, the almost identical plasma concentrations and time-courses of phylloquinone epoxide suggested an apparent equal effectiveness for the enantiomers. It is probable, however, that the similar plasma concentrations of phylloquinone epoxide reflected the saturation kinetics found for racemic warfarin (Fig. 2) and that the dose of each enantiomer exceeded that required for the maximal accumulation of phylloquinone epoxide.

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