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WARFARIN AND METABOLISM OF VITAMIN K_1

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Abstract—To further examine the hypothesis that warfarin inhibits prothrombin synthesis by interfering with the cyclic interconversion of vitamin K_1 and phylloquinone epoxide, the metabolism of tracer doses of labeled vitamin K_1 was studied in anticoagulant-treated rats. A tracer dose of [^{3}H]K_1 initially turned over with a half-life of 2·2 hr in the liver, but after 5 hr the degradation rate decreased considerably. Warfarin caused hepatic [^{3}H]K_1 levels to drop to 50 per cent of controls 5 min after administration of the vitamin, and over 10 hr the concentration of [^{3}H]K_1 was 26–36 per cent of control levels. This decrease in vitamin K_1 was not large enough to account for the inhibition of prothrombin synthesis by warfarin. Thirty-five min after warfarin administration, there was more [^{3}H]phylloquinone epoxide in the liver than labeled vitamin and over 10 hr the [^{3}H]epoxide:[^{3}H]K_1 ratio varied from 1·6 to 3·0. In addition to increasing the relative amount of [^{3}H]epoxide, warfarin and phenylindanedione also increased hepatic metabolites more polar than vitamin K_1 or epoxide. However, warfarin did not increase plasma or urinary radioactivity. The urine was the principal excretory route for metabolites of vitamin K_1. 2-Diethylaminoethyl-2,2, diphenylvalerate hydrochloride (SKF 525-A) inhibited the turnover of [^{3}H]K_1 but did not decrease the [^{3}H]polar metabolites in the liver in control or warfarin-treated rats. The drug also did not inhibit the epoxidation of the vitamin to phylloquinone epoxide. The metabolism of vitamin K_1 was not altered by vitamin K deficiency or chronic administration of the vitamin. After a small dose of warfarin (35 μg/100 g body wt), plasma prothrombin decreased for 14 hr and then slowly rose but did not reach 90 per cent of normal until 3 days after administration of anticoagulant. The epoxide:K_1 ratio in the liver, measured by injecting a tracer dose of [^{3}H]K_1, was 0·90 at 6 hr when prothrombin synthesis was completely blocked. The ratio dropped to 0·52 just before prothrombin started to rise at 14 hr and remained around 0·5 during the slow climb of plasma prothrombin toward the normal level. These low ratios were unexpected in animals in which prothrombin synthesis was partially or completely blocked. Hepatic epoxide:K_1 ratios of 11·4 and 1·3 were observed in warfarin-treated rats in which prothrombin synthesis was stimulated by injections of 0·1 mg [^{3}H]K_1 plus 0·4 mg [^{3}H]epoxide and 0·1 mg [^{3}H]K_1 respectively. Therefore, there appears to be a lack of correlation of epoxide:K_1 ratios and prothrombin synthesis.

WE HAVE proposed that coumarin and indanedione anticoagulants exert their effects by inhibiting the conversion of phylloquinone epoxide, a metabolite of vitamin K_1, back to the vitamin and causing the accumulation of this inhibitor of the vitamin.[1–6] This is supported by the observation that, in warfarin-resistant rats, the epoxide to K_1 conversion was not inhibited by warfarin or phenylindanedione except at high doses which also inhibited prothrombin synthesis.[5,6] This theory of action requires that the endogenous epoxide:K_1 ratio be elevated to an inhibitory ratio over the time when the anticoagulant is blocking prothrombin synthesis and the ratio be reduced as prothrombin synthesis is restored to normal. Also the elevation of the epoxide:K_1 ratio should occur as rapidly as warfarin is observed to block clotting protein synthesis.

In previous studies, warfarin caused a decrease in hepatic levels of radioactive vitamin K_1 as compared to controls after 100 μg doses of [^{3}H]K_1.[5] Studies with tracer amounts of vitamin K would determine whether warfarin lowers endogenous levels of the vitamin sufficiently to be important in its anticoagulant action. A 10-g rat liver contains approximately 0·7 μg vitamin K as estimated by chick bioassay.[7] In the following studies, 5 ng/100 g body wt of [^{3}H]K_1 was injected as a tracer dose.

Recently Shearer *et al.*[8] observed that [^{3}H]epoxide accumulated in the plasma of warfarin-treated patients injected with [^{3}H]K_1, indicating that warfarin has a similar effect in humans as in rats and rabbits. They also observed that the anticoagulant increased plasma and urinary radioactivity. Since we found in preliminary studies that warfarin increased the amount of polar metabolites of [^{3}H]K_1 in rat liver, we studied this additional effect of warfarin further.[9]

MATERIALS AND METHODS

Ten- to 15-week-old male Sprague–Dawley rats (Charles River Laboratories, CD strain) were used in these experiments. 6, 7-[^{3}H]K_1 and [phytyl-^{14}C]K_1 were synthesized by J. T. Matschiner and C. Siegfried (Biochemistry Dept., Univ. of Nebraska School of Medicine, Omaha, Nebr.) and purified as described previously.[1] The labeled vitamin was dissolved in Tween 80 and diluted with 0·9% NaCl to make solutions containing 5% Tween or less; 0·1 ml was injected intracardially. Phenylindanedione (K & K Laboratories) was also dissolved in Tween 80 and enough water added to make a solution 10 per cent in Tween and containing 3 mg/ml of the drug. Sodium warfarin and 2-diethylaminoethyl-2,2, diphenylvalerate hydrochloride (SKF 525-A) were kindly provided by Endo Laboratories and Smith, Kline & French respectively. Plasma prothrombin was assayed by the method of Hjort *et al.*,[10] using a Fibrometer coagulation timer (Bioquest, Cockeysville, Md.). Control plasma was pooled plasma from twenty 11- to 12-week-old male rats. The results are expressed as per cent of control prothrombin, although the assay is somewhat sensitive to the concentration of Factor X which is also a vitamin K-dependent factor.

RESULTS

Warfarin and the hepatic metabolism of [^{3}H]K_1

The decline of radioactivity in livers of rats injected with a tracer dose of [^{3}H]K_1 was rapid over the first 5 hr and then decreased more slowly (Fig. 1). In warfarin-treated rats, the turnover was similar, but the hepatic radioactivity was slightly higher at 2, 5 and 10 hr after administration. The effect of warfarin was not on the uptake, since the liver radioactivity was similar 5 min after administration in warfarin-treated and control rats.

Hepatic [^{3}H]K_1 decreased logarithmically with a half-life of about 2·2 hr in the control and 1·8 hr in the warfarin-treated group. After 5 hr, the concentration decreased at a slower rate.

Warfarin and hepatic concentration of [^{3}H]K_1, [^{3}H]*epoxide and* [^{3}H]*polar metabolites*

Warfarin caused a rapid decrease in hepatic [^{3}H]K_1 (Fig. 1). As early as 5 min after administration, the concentration of vitamin was about half the control, and

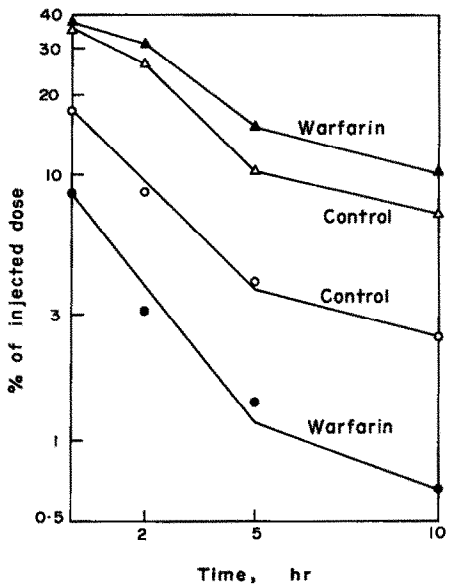

FIG. 1. Warfarin and turnover of hepatic radioactivity and $[^3H]K_1$. Control rats and rats injected with warfarin (0·1 mg/100 g body wt) intraperitoneally were injected intracardially with $[^3H]K_1$ (5 ng/100 g body wt) and killed at 5 min, 2, 5 and 10 hr after administration of the vitamin. Livers were analyzed as described previously[1,5] for radioactivity (△, control; ▲, warfarin) and $[^3H]K_1$ (○, control; ●, warfarin). Each value is the average for three to ten rats.

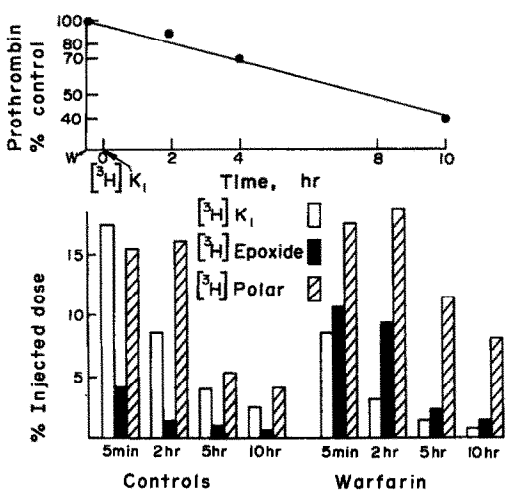

FIG. 2. Warfarin and metabolism of $[^3H]K_1$. Control rats and rats injected with warfarin (0·1 mg/100 g body wt) intraperitoneally 0·5 hr before were injected intracardially with $[^3H]K_1$ (5 ng/100 g body wt) and killed at times indicated after vitamin administration. Livers were analyzed as described previously.[1,5] The $[^3H]$polar metabolites were the hepatic ^3H not extracted by hexane plus the radioactivity which was eluted after $[^3H]K_1$ and $[^3H]$epoxide from silicic acid columns. No $[^3H]$metabolites less polar than the vitamin or epoxide were detected. Each value is the average for three to ten rats. The upper curve shows plasma prothrombin concentrations[10] at 2, 5 and 10 hr after warfarin administration. The arrows indicate the times at which $[^3H]K_1$ and warfarin (W) were injected.

after this time it was 26–36 per cent of control levels. Much of the loss of $[^3H]K_1$ was due to its oxidation to phylloquinone epoxide whose conversion back to vitamin K_1 was blocked by warfarin. Five min after administration of vitamin, there was more $[^3H]$epoxide in the liver than $[^3H]K_1$ and after this time the epoxide:K_1 ratio varied from 1·6 to 3·0 (Fig. 2). The ratios in livers of control rats were all around 0·2. In addition to increasing the relative amount of epoxide, warfarin also increased metabolites more polar than the vitamin and epoxide. At 5 min and 2 hr after administration of $[^3H]K_1$, the polar metabolites in the livers of warfarin-treated rats were about the same as controls, but at 5 and 10 hr the polar metabolites were twice those in controls.

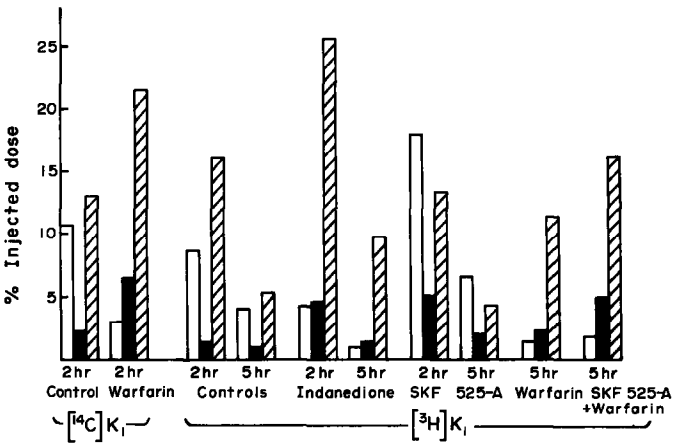

FIG. 3. Effect of anticoagulants and SKF 525-A on metabolism of $[^3H]K_1$ and [phytyl-^{14}C]K_1. Groups of control rats or groups injected with warfarin (0·1 mg/100 g body wt) or phenylindanedione (1 mg/100 g body wt) 0·5 hr before were injected intracardially with [phytyl-^{14}C]K_1 (300 ng/100 g body wt) or $[^3H]K_1$ (5 ng/100 g body wt). Groups treated with SKF 525-A were injected intraperitoneally with the drug (5 mg/100 g body wt) at 0·5 hr before and 2·5 hr after administration of vitamin. Livers were removed at times indicated and analyzed as described previously.[1,5] The results are presented as in Fig. 2. Each value is the average for three or more rats.

The effect of warfarin on the metabolism of $[^{14}C]K_1$, which is uniformly labeled in the phytyl side chain rather than in the napthoquinone ring where $[^3H]K_1$ is labeled, was also studied (Fig. 3). The anticoagulant increased the amount of polar metabolites from $[^{14}C]K_1$ in the liver by 65 per cent at 2 hr. Phenylindanedione, an antagonist of vitamin K whose mechanism of action appears to be similar to warfarin,[6] also increased the amount of polar metabolites by 56 and 81 per cent at 2 and 5 hr after $[^3H]K_1$ administration. In contrast, 2-chloro-3-phytyl-napthoquinone and tetrachloropyridinol, antagonists of vitamin K whose inhibition of clotting protein synthesis appears to be at different sites than the coumarins and indanediones,[6] did not increase the concentration of hepatic polar metabolites.

SKF 525-A and metabolism of $[^3H]K_1$

The increase in polar metabolites caused by warfarin could be due to induction of microsomal enzymes which possibly metabolize vitmin K_1. A number of coumarin derivatives induce microsomal oxidases.[11] To determine whether vitamin K_1 is

metabolized to a significant extent by microsomal oxidases, the effect of SKF 525-A, an inhibitor of these enzymes,[12] on the metabolism of the vitamin was studied (Fig. 3). The drug apparently did not affect the epoxidation of vitamin K_1 to phylloquinone epoxide since the [^{3}H] epoxide:[^{3}H]K_1 ratios were similar to controls at 2 and 5 hr. SKF 525-A did inhibit the turnover of vitamin K_1, since the hepatic concentrations of [^{3}H]K_1 were 107 and 62 per cent greater than controls at 2 and 5 hr respectively. However, the amounts of [^{3}H]polar metabolites in the livers of SKF 525-A-treated rats were similar to controls. When warfarin-treated rats were also injected with SKF 525-A, the metabolism of [^{3}H]K_1 was little different than with warfarin alone (Fig. 3). Again, SKF 525-A apparently did not inhibit epoxidation, since the [^{3}H]epoxide:[^{3}H]K_1 ratio was 2·7 at 5 hr in rats treated with the drug and warfarin (Fig. 3). The amount of remaining [^{3}H]K_1 was slightly higher, but the polar metabolites were not decreased in the warfarin plus SKF 525-A group as compared to rats treated only with warfarin.

Time required for warfarin to block prothrombin synthesis and increase hepatic epoxide:K_1 ratio

After warfarin administration, plasma prothrombin decreased logarithmically from zero time, but a delay of up to 1 hr in inhibition of prothrombin synthesis would probably not be detected (Fig. 2). The hepatic [^{3}H]epoxide:[^{3}H]K_1 ratio was elevated to 1·25, which was about 7-fold greater than controls at 35 min after warfarin administration (5 min after the injection of [^{3}H]K_1) which is consistent with the rapid onset of warfarin inhibition (Fig. 2). In a further experiment, three rats injected 5 min previously with a tracer dose of [^{3}H]K_1 were given warfarin (0·1 mg/100 g body wt) intraperitoneally and killed 10 min after the anticoagulant. Liver analysis showed that [^{3}H]epoxide:[^{3}H]K_1 ratio had increased to 0·52 ± 0·09 (about 3-fold greater than controls) within 10 min after warfarin.

Effect of warfarin on plasma and urinary radioactivity after [^{3}H]K_1 administration

Since warfarin caused an increase in the polar metabolites of vitamin K_1 in the liver, we determined the effect on plasma and urinary metabolites. Excretion in the urine appeared to be the principal excretory route of [^{3}H]K_1, as about 60 per cent of the dose was found in the urine over 3 days after administration of the vitamin (Table 1). Warfarin had no effect on the excretion rate. The decrease in plasma radioactivity was rapid over the first 10 hr and then slowed down (Fig. 4). The anticoagulant had little effect on the turnover rate.

TABLE 1. EXCRETION OF ^{3}H IN URINE AFTER [^{3}H]K_1 INJECTION*

	% Injected ^{3}H					
	0–10 hr	10–20 hr	20–30 hr	30–48 hr	48–72 hr	Total 0–72 hr
Control	36·9	8·0	5·8	6·1	3·0	59·8
Warfarin	36·7	11·6	6·6	4·9	2·0	61·8

* Two pairs of control rats and two pairs injected with warfarin (0·1 mg/100 g body wt) 0·5 hr before were injected intracardially with [^{3}H]K_1 (5 ng/100 g body wt) and placed in four metabolism cages. Urine was collected over the periods indicated and the ^{3}H assayed. Each value is the average from two metabolism cages.

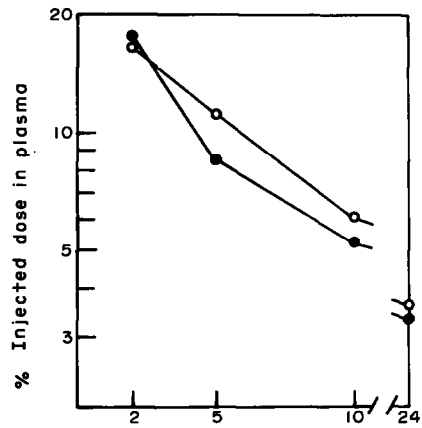

FIG. 4. Plasma radioactivity in warfarin-treated rats. Control rats (○) and rats injected with warfarin (0·1 mg/100 g body wt) intraperitoneally 0·5 hr previously (●) were injected intracardially with [^{3}H]K_1 (5 ng/100 g body wt). Blood samples were taken from the heart at times indicated and plasma radioactivity was assayed. Each value is the average for four rats.

Effect of chronic vitamin K administration and vitamin K deficiency on metabolism of [^3H]K_1

Since vitamin K deficiency caused vitamin K_1 epoxidase activity to increase,[13] rats fed a vitamin K-deficient diet for 7 days resulting in an average prothrombin concentration of 45 per cent of normal were injected with a tracer dose of [^{3}H]K_1 and livers were analyzed 2 hr later. The [^{3}H]K_1:[^{3}H]epoxide ratio and the amount of unmetabolized [^{3}H]K_1 were similar to control values. Since chronic administration of many drugs increases their turnover rate, another group of rats injected daily for 7 days with 1 mg vitamin K_1 intramuscularly were injected with a tracer dose of [^{3}H]K_1 24 hr after the last dose of vitamin and killed 5 hr later. Liver analysis revealed that the metabolism was little different than that of controls.

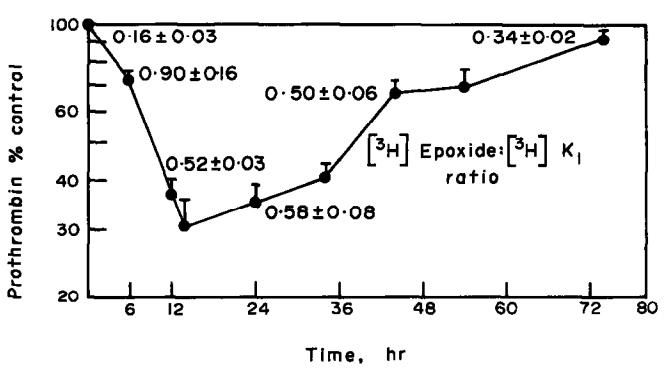

FIG. 5. Metabolism of [^{3}H]K_1 during recovery from warfarin. Groups of rats injected with warfarin (35 μg/100 g body wt) at zero time were injected with [^{3}H]K_1 (5 ng/100 g body wt) intracardially at 2 hr before zero time and at 4, 10, 22, 42 and 72 hr after zero time. Each group was killed 2 hr after injection of the vitamin, the livers were removed and the [^{3}H]epoxide:[^{3}H]K_1 was determined.[1,5] Each ratio is the average for three to six rats ± S. E. shown in the figure. Blood samples were taken at times indicated for prothrombin assays. Each point is the average for seven to twenty rats with the S. E. shown.

Prothrombin synthesis and hepatic epoxide:K_1 ratios during recovery from warfarin

To determine if there is a correlation between the epoxide:K_1 ratios and inhibition of prothrombin synthesis, these parameters were measured during recovery from a small dose of warfarin (35 μg/100 g body wt). Plasma prothrombin decreased for 14 hr, after which it slowly increased but did not reach 90 per cent of normal until 3 days after administration of warfarin. The ratio of [^{3}H]epoxide to [^{3}H]K_1 was determined by injecting a tracer dose of [^{3}H]K_1 2 hr before each point indicated (Fig. 5) and analyzing the livers 2 hr later. The ratio was increased to greater than five times the control at 6 hr at which time prothrombin synthesis could be assumed to be almost completely blocked. At 12 hr, the ratio had dropped to about three times greater than control. After 12 hr the epoxide:K_1 ratio remained around 0·5 while prothrombin increased slowly from 14 to 72 hr at a rate much less than the theoretical increase which would have occurred if there was no inhibition of prothrombin synthesis.[14] This suggests that epoxide:K_1 ratios as low as 0·5 are correlated with partial inhibition of prothrombin synthesis. This was surprising in view of our previous study in which phylloquinone epoxide inhibited the stimulation of prothrombin synthesis by vitamin K_1 in warfarin-treated rats.[3] An administered epoxide:K_1 ratio of 8:1 was required to inhibit the response to the vitamin by about 60 per cent. Since the epoxide:K_1 ratio in the liver was not determined in the earlier experiments, we administered 0·1 mg [^{3}H]K_1 and 0·4 mg [^{3}H]epoxide to warfarin-treated rats and removed the livers 1 hr later for analysis. Another group was injected with 0·1 mg [^{3}H]K_1 only and killed at 1 hr. The response to [^{3}H]K_1 alone was as

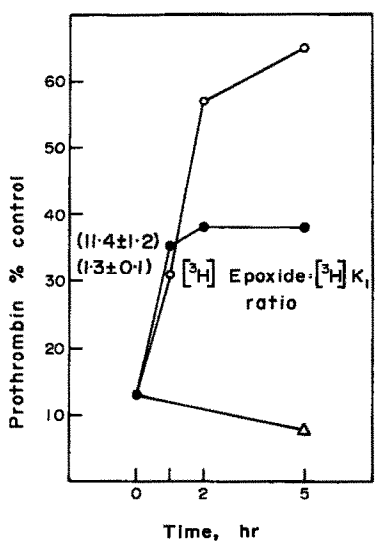

FIG. 6. Hepatic [^{3}H]epoxide:[^{3}H]K_1 ratios in warfarin-treated rats responding to K_1 and K_1 plus epoxide. Rats injected intraperitoneally with warfarin (0·1 mg/100 g body wt) 25 hr before were divided into five groups. Group I was injected intracardially with 0·1 mg K_1 (○), Group II with 0·1 mg of K_1 + 0·4 mg epoxide (●) and Group III was untreated (△). Blood samples were taken at 1, 2 and 5 hr for prothrombin assay. Group IV was injected intracardially with 0·1 mg [^{3}H]K_1 (○) and Group V with 0·1 mg [^{3}H]K_1 plus 0·4 mg [^{3}H]epoxide (●). The [^{3}H]K_1 and [^{3}H]epoxide had the same specific activity (3×10^6 dis/min/mg). Blood samples were taken and the livers removed at 1 hr for analysis.[1,5] The hepatic [^{3}H]epoxide: [^{3}H]K_1 ratios with the S.E. are shown. Each value is the average for three livers. The prothrombin concentrations are the averages for four to nine animals.

rapid over 2 hr as observed in vitamin K-deficient rats injected with vitamin K_1 (Fig. 6). At 1 hr, the hepatic [^{3}H]K_1:[^{3}H]epoxide ratio was 1·3. The group treated with [^{3}H]K_1 and [^{3}H]epoxide in a 1:4 ratio showed a partial response and the ratio was 11·4 at 1 hr.

DISCUSSION

The turnover of [^{3}H]K_1 in the liver after a tracer dose was not greatly different from that found when a dose two orders of magnitude higher was administered.[15] Ten hr after injection of 15 ng or 3 μg [^{3}H]K_1, 3 and 5 per cent, respectively, of the injected radioactivity in the liver was radioactive vitamin (see Fig. 1 and Ref. 15). Two hr after administration of 15 ng or 100 μg [^{3}H]K_1, the per cent of the injected dose which was hepatic [^{3}H]K_1 was 9 and 10 per cent, respectively (see Fig. 1 and Ref. 4). These data suggest that the turnover of vitamin K_1 in the liver may not be greatly influenced by the amount administered if the doses are below several hundred micrograms. The decline in hepatic [^{3}H]K_1 in this study was biphasic and resembled that observed by Matschiner[15] who found that the initial half-life of [^{3}H]K_1 was about 2 hr with a subsequent slowing down of metabolism. The initial turnover rate of [^{3}H]K_1 estimated from the present study ($T_{\frac{1}{2}}$ = 2·2 hr) is probably low because after intracardial injection of radioactive vitamin the hepatic level of [^{3}H]K_1 does not reach a maximum until about 0·5 hr after administration.[15] In any case, the metabolism of vitamin K_1 in the liver is rapid and consistent with the rapid onset of vitamin K deficiency in male rats.

Warfarin did not increase the excretion of labeled metabolites of [^{3}H]K_1 in plasma and urine of the rat as Shearer *et al.*[8] had observed in humans, but warfarin and phenylindanedione clearly increased the amount of polar metabolites of both [^{3}H]K_1 and [^{14}C-phytyl]K_1 in rat liver. In clinical studies, warfarin caused an increase in plasma radioactivity after [^{3}H]K_1 administration which was due mainly to the accumulation of [^{3}H]phylloquinone epoxide. The anticoagulant also increased the urinary excretion of water-soluble metabolites of the vitamin.[8] We also observed an accumulation of [^{3}H]epoxide in the plasma of warfarin-treated rats,* but the total radioactivity in the plasma was the same as that of controls (Fig. 4). Thus in both humans and rats, oral anticoagulants caused a relative increase of phylloquinone epoxide and metabolites of the vitamin more polar than the epoxide. The possibility that the metabolism of vitamin K may have been stimulated by microsomal oxidases induced by warfarin is unlikely, since the amounts of hepatic [^{3}H]polar metabolites were little affected by SKF 525-A. SKF 525-A inhibited the hepatic turnover of vitamin K_1 suggesting that the vitamin is metabolized by microsomal oxidases. The drug, however, had little effect on the epoxide:K_1 ratio, indicating that the epoxidation of vitamin K_1 is not dependent on P-450 linked microsomal oxidases which is consistent with the observations of Willingham and Matschiner[16] on the activity of vitamin K_1 epoxidase *in vitro*.

Studies in rat and man indicated that the chief excretory route for large doses of vitamin K_1 was intestinal excretion.[17–19] The present study suggests that physiological amounts of vitamin K_1 are eliminated mainly through the urine.

* P. Ren and R. G. Bell, unpublished results.

Warfarin (0·1 mg/100 g body wt) rapidly affected the K_1-epoxide interconversion, as the epoxide:K_1 ratio was three times that of controls 10 min after administration and seven times greater at 35 min where the ratio was 1·2. The ratio remained above 1·2 over 10 hr, during which time prothrombin synthesis was completely blocked. The conversion of K_1 to epoxide is relatively rapid, since in warfarin-treated animals there was more labeled epoxide than vitamin in the liver as early as 5 min after administration of vitamin. In warfarin-treated rats [^{3}H]epoxide accounted for 14–30 per cent of the hepatic radioactivity, indicating that the K_1 to epoxide conversion is a major metabolic pathway for the vitamin. Although warfarin lowered the amount of [^{3}H]K_1 as low as 26 per cent of control levels, this is not likely to be the principal mechanism of action of the anticoagulant since as much as 8 μg/100 g body wt of vitamin K_1 is ineffective in overcoming warfarin inhibition of prothrombin synthesis[5] and less that 0·2 μg/100 g body wt of vitamin is required to stimulate prothrombin synthesis in vitamin K-deficient rats.[20]

In rats treated with a low dose of warfarin (35 μg/100 g body wt), the hepatic epoxide:K_1 ratio was 0·9 at 6 hr when prothrombin synthesis was completely blocked and 0·5 from 14 to 72 hr when prothrombin synthesis was partially blocked (Fig. 5). In contrast, the ratios were 1·3 and 11·4 in rats in which plasma prothrombin was rapidly increasing (Fig. 6). This suggests that high hepatic epoxide:K_1 ratios are not correlated with inhibition of prothrombin synthesis, at least in animals given large doses of the vitamin. This lack of correlation may be due to the whole liver ratio not being the same as that at the site of vitamin K action. However, the observation of an increase in plasma prothrombin when the epoxide:K_1 ratio was 11·4 makes this an unlikely explanation. Perhaps the epoxide:K_1 ratio is less important than the interruption of the cyclic interconversion of K_1 and phylloquinone epoxide. We have recently found that the anticoagulants chloro-vitamin K_1 and tetrachloropyridinol interrupt the K_1-epoxide cycle by inhibiting the epoxidase step.[21] The involvement of the K_1-epoxide cycle in the mechanism of action of warfarin seems very likely, since in coumarin-resistant rats the effect of the anticoagulant on this cycle was greatly reduced.[5] However, the hypothesis that warfarin inhibits clotting protein synthesis by causing the accumulation of epoxide does not appear to be tenable.[3]

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REFERENCES

1. J. T. Matschiner, R. G. Bell, J. M. Amelotti and T. E. Knauer, *Biochim. biophys. Acta* **201**, 309 (1970).
2. R. G. Bell and J. T. Matschiner, *Archs Biochem. Biophys.* **141**, 473 (1970).
3. R. G. Bell and J. T. Matschiner, *Nature, Lond.* **237**, 32 (1972).
4. R. G. Bell, J. A. Sadowski and J. T. Matschiner, *Biochemistry, N.Y.* **11**, 1959 (1972).
5. R. G. Bell and P. T. Caldwell, *Biochemistry, N.Y.* **12**, 1759 (1973).
6. P. Ren, R. E. Laliberte and R. G. Bell, *Molec. Pharmac.* **10**, 373 (1974).
7. J. T. Matschiner and E. A. Doisy Jr., *J. Nut.* **90**, 97 (1966).
8. M. J. Shearer, A. McBurney and P. Barkhan, *Br. J. Haemat.* **24**, 471 (1973).
9. R. G. Bell and P. Caldwell, *Int. Congress Biochem.* Stockholm, Section 9f 12 (1973).
10. P. Hjort, S. I. Rapaport and P. A. Owren, *J. Lab. clin. Med.* **46**, 89 (1955).
11. G. Feuer, *Can. J. Physiol. Pharmac* **48**, 232 (1970).
12. A. H. Conney, in *Fundamentals of Drug Metabolism and Drug Disposition* (Eds. B. N. Ladu, H. G. Mandel and E. L. Way), p. 269. Williams & Wilkins, Baltimore (1971).
13. A. K. Willingham and J. T. Matschiner, *Int. Congress Biochem.*, Stockholm, Section 7b 9 (1973).
14. R. G. Bell and J. T. Matschiner, *Archs. Biochem. Biophys.* **135**, 152 (1969).

15. J. T. MATSCHINER, in *Fat-Soluble Vitamins* (Eds. H. F. DELUCA and J. W. SUTTIE), p. 377. Univ. of Wisconsin Press, Madison (1970).
16. A. K. WILLINGHAM and J. T. MATSCHINER, *Fedn Proc.* **31,** 1305 (1972).
17. J. D. TAYLOR, G. J. MILLAR, L. B. JAQUES and J. W. T. SPINKS, *Can. J. Biochem. Physiol.* **34,** 1143 (1956).
18. O. WISS and H. GLOOR, *Vitams Hor.* **24,** 575 (1966).
19. M. J. SHEARER, C. N. MALLINSON, G. R. WEBSTER and P. BARKHAN, *Br. J. Haemat.* **22,** 579 (1972).
20. J. T. MATSCHINER and W. V. TAGGART, *J. Nutr.* **94,** 57 (1968).
21. A. K. WILLINGHAM, R. E. LALIBERTE, R. G. BELL and J. T. MATSCHINER, *Fedn Proc.* **22,** 672 (1974).