MOLECULAR PHARMACOLOGY, **10**, 373–380

# Effects of Warfarin, Phenylindanedione, Tetrachloropyridinol, and Chloro-vitamin $K_1$ on Prothrombin Synthesis and Vitamin K Metabolism in Normal and Warfarin-Resistant Rats

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(Received October 23, 1973)

SUMMARY

REN, PETER, LALIBERTE, RONALD E., AND BELL, ROBERT G.: Effects of warfarin, phenylindanedione, tetrachloropyridinol, and chloro-vitamin $K_1$ on prothrombin synthesis and vitamin K metabolism in normal and warfarin-resistant rats. *Mol. Pharmacol.* **10,** 373–380 (1974).

Anatagonists of vitamin K—warfarin, phenylindanedione the 2-chloro analogue of vitamin $K_1$, and tetrachloropyridinol—blocked prothrombin synthesis completely and without a lag period in the rat. Experiments to determine the mode of action of the antagonists indicated that they could be divided into two groups. The inhibition of prothrombin synthesis by all four antagonists was reversed by vitamin $K_1$, but the inhibition in Sprague-Dawley rats by group I (warfarin and phenylindanedione) was not reversed by phylloquinone epoxide, because the group I compounds inhibited the conversion of [$^3$H] phylloquinone epoxide to the active vitamin, [$^3$H]vitamin $K_1$, and caused the accumulation of [$^3$H] phylloquinone epoxide in rats that had received injections of the tritiated vitamin. It is proposed that group I compounds were ineffective in blocking prothrombin synthesis in warfarin-resistant rats because in these animals the conversion from epoxide to $K_1$ is not as sensitive as in normal animals. Consequently the epoxide can stimulate prothrombin synthesis in the presence of group I compounds in the resistant strain. In contrast, the inhibition of prothrombin synthesis by group II (tetrachloropyridinol and 2-chloro-vitamin $K_1$) was reversed by epoxide, because these compounds inhibited the [$^3$H] phylloquinone epoxide to [$^3$H] vitamin $K_1$ conversion much less than those of group I. Similarly, the effect of group II on the metabolism of [$^3$H] vitamin $K_1$ was much smaller than that of group I. Group II compounds blocked prothrombin synthesis in warfarin-resistant animals at least as well as in Sprague-Dawley rats. These results are consistent with the idea that coumarin and indanedione anticoagulants (group I) inhibit prothrombin synthesis by causing the accumulation of an inhibitor of the vitamin, phylloquinone epoxide, and that 2-chloro-vitamin $K_1$ and tetrachloropyridinol (group II) act at another site.

## INTRODUCTION

The discovery that warfarin profoundly influences the metabolism of vitamin $K_1$, leading to the accumulation of a metabolite of the vitamin, phylloquinone epoxide (1, 2),

This work was supported by grants from the National Heart and Lung Institute (HL 14847) and from the Research Committee of the University of Rhode Island. A preliminary account of this work was presented at the 1973 meeting of the Federation of American Societies for Experimental Biology and Medicine. (16).

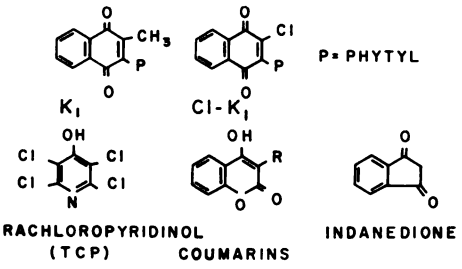


FIG. 1. *Structures of vitamin K antagonists*

prompted us to examine the effects of other vitamin K antagonists on the metabolism of the vitamin and the regulation of prothrombin synthesis. Outside of the coumarins, the most powerful antagonists of vitamin K are the indanediones, 2-chloro-3-phytylnaphthoquinone and tetrachloropyridinol (Fig. 1). The coumarins and indanediones have been used clinically in treating thromboembolic disease, while the chloro analogue of vitamin $K_1$ has been used in studies of the mechanism of action of the vitamin (3). Tetrachloropyridinol was discovered only recently to have anticoagulant activity and to be antagonistic to vitamin K (4, 5).

It has been demonstrated recently that warfarin causes the accumulation of a microsomal protein which can be converted to thrombin by *Echis carinatus* venom (6, 7). This suggests that warfarin blocks the vitamin K-dependent conversion of this protein to active prothrombin (6). We have proposed that warfarin inhibits the production of prothrombin by causing the accumulation of phylloquinone epoxide, an inhibitor of the vitamin (2, 8). Vitamin $K_1$ is converted to the epoxide by a microsomal epoxidase (9), and the epoxide is converted back to the vitamin by a microsomal reductase which is inhibited strongly by warfarin (10). In rats genetically resistant to the coumarin anticoagulants the conversion of epoxide to $K_1$ is less sensitive to inhibition by warfarin and the epoxide does not accumulate (10, 11). We have compared the effects of the other vitamin K antagonists with those of warfarin on the synthesis of prothrombin and the metabolism of radioactive vitamin $K_1$ and phylloquinone epoxide in Sprague-Dawley and warfarin-resistant rats.

## MATERIALS AND METHODS

[6, 7-$^3$H] Vitamin $K_1$ and tritiated phylloquinone epoxide were generous gifts from J. T. Matschiner and C. Siegfried (Biochemistry Department, University of Nebraska School of Medicine, Omaha) and were purified as described previously (1). 2-Chlorovitamin $K_1$ was kindly provided by J. Lowenthal (Pharmacology Department, McGill University, Montreal) and was purified by chromatography on silicic acid (1). The sodium salt of tetrachloropyridinol was a gift from F. Marshall (Dow Chemical Company, Zionsville, Ind.), and sodium warfarin, from Endo Laboratories (Garden City, N. Y.). 2-Phenyl-1,3-indanedione was purchased from K & K Laboratories. Unlabeled phylloquinone epoxide was prepared according to Tishler *et al.* (12). [$^3$H]Vitamin $K_1$, [$^3$H]phylloquinone epoxide, unlabeled $K_1$, unlabeled epoxide, and 2-chloro-vitamin $K_1$ were dissolved in Tween 80 and diluted with 0.9% NaCl to make solutions containing 5% Tween or less; 0.1 or 0.2 ml was injected intracardially or intramuscularly. Phenylindanedione was also dissolved in Tween 80, and enough water was added to make a solution 10% in Tween and containing about 3 mg/ml of the antagonist. Sodium warfarin and sodium tetrachloropyridinol were dissolved in water.

Warfarin-resistant male rats, obtained as described previously (11), and male Sprague-Dawley rats (10–15 weeks old) were used in these experiments.

Plasma prothrombin was assayed by the method of Hjort *et al.* (13). Control plasma was pooled plasma from twenty 11–12-week-old male Sprague-Dawley rats. The results are expressed as a percentage of control prothrombin, although the assay is somewhat sensitive to the concentration of factor X, which is also a vitamin K-dependent factor.

## RESULTS

*Inhibition of prothrombin synthesis by vitamin K antagonists.* Marshall (4) found that an intragastric dose of tetrachloropyridinol of 4–8 mg/100 g of body weight was maximally effective in increasing prothrombin times 24 hr after administration.

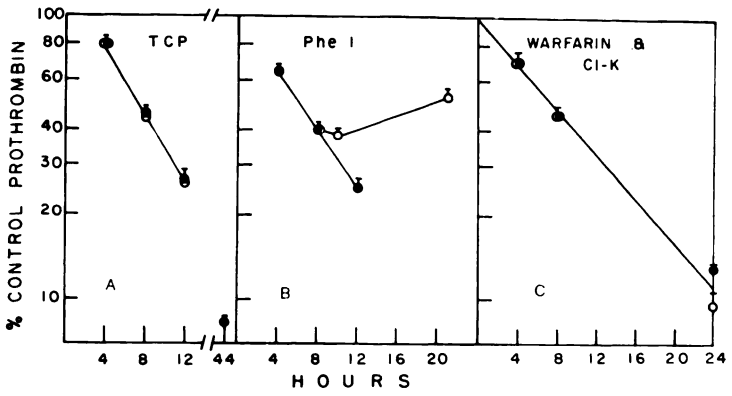


FIG. 2. *Effects of tetrachloropyridinol (TCP) (A), phenylindanedione (Phe I) (B), and warfarin and 2-chloro-vitamin $K_1$ (C) on plasma prothrombin*

A. Rats were given an intraperitoneal injection of tetrachloropyridinol (○, 4 mg/100 g; ●, 6 mg/100 g). Each point is the mean for three rats, with the vertical lines indicating the standard error.

B. Rats were given an intraperitoneal injection of phenylindanedione (1 mg/100 g) at zero time only (○) or at zero time and 8 hr (●). Each point is the mean for three rats, with the vertical lines indicating the standard error.

C. Rats were given an intraperitoneal injection of warfarin (●, 0.1 mg/100 g) or an intracardiac injection of 2-chloro-vitamin $K_1$ (○, 0.25 mg/100 g). Each point is the mean for four rats, with the vertical lines indicating the standard error.

After intraperitoneal injection of tetrachloropyridinol (4 or 6 mg/100 g) plasma prothrombin decreased with a half-life of 7.0 hr (Fig. 2A). After this time the rate of loss decreased, but low prothrombin levels were found 44 hr after administration. Administration of phenylindanedione (1 mg/100 g) caused prothrombin to decrease logarithmically, with a half-life of 6.0 hr (Fig. 2b). To extend the exponential decay past 8 hr, a second dose of phenylindanedione was required. This is consistent with the rapid disappearance of plasma phenylindanedione relative to coumarin anticoagulants observed by Millar *et al.* (14). For comparison, the half-life of prothrombin after administration of warfarin or 2-chloro-vitamin $K_1$ was 7.5 hr (Fig. 2C). This suggests that the four antagonists block prothrombin synthesis completely without a substantial lag period.

*Reversal of inhibition of prothrombin synthesis by vitamin $K_1$.* It is well known that inhibition of prothrombin synthesis by warfarin or 2-chloro-vitamin $K_1$ can be reversed by vitamin $K_1$ (3). In rats treated with phenylindanedione at zero time and after 8 hr, vitamin $K_1$ injected after 12 hr produced an increase in plasma prothrombin levels (Fig. 3A). Similarly, in rats treated with tetrachloropyridinol 24 hr previously, the vitamin also reversed the inhibition of prothrombin synthesis (Fig. 3B). Marshall (5) also found that if vitamin $K_1$ was administered to rats along with tetrachloropyridinol, the prothrombin time returned to normal more rapidly than with tetrachloropyridinol alone.

*Effect of phylloquinone epoxide on inhibition of prothrombin synthesis.* Since phylloquinone epoxide was ineffective against warfarin because the anticoagulant inhibits its conversion to vitamin $K_1$ (15), it was tested against the other anticoagulants. The epoxide was much less effective than vitamin $K_1$ in overcoming the inhibition of prothrombin synthesis by phenylindanedione (Fig. 3A) but was as potent as the vitamin in stimulating prothrombin synthesis in animals treated with either tetrachloropyridinol or 2-chloro-vitamin $K_1$ (Fig. 3B and C).

*Effect of anticoagulants on plasma prothrombin in warfarin-resistant rats.* To test the effect of other anticoagulants on warfarin-resistant rats, males were treated with vitamin $K_1$ (25 μg/100 g) in order to increase plasma prothrombin levels to normal. Resistant rats have prothrombin levels of 17 ±

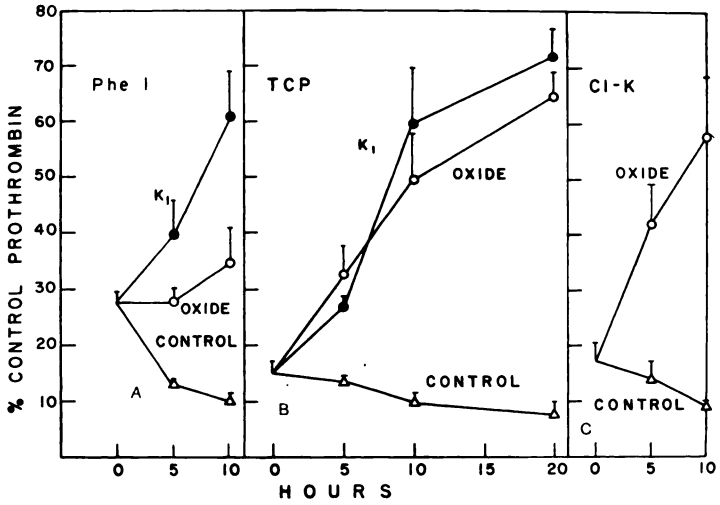


FIG. 3. *Inhibition of prothrombin synthesis and reversal by $K_1$ and epoxide*

A. Effect of vitamin $K_1$ and phylloquinone epoxide on inhibition by phenylindanedione (Phe I). Rats received phenylindanedione (1 mg/100 g) intraperitoneally at zero time and 8 hr. At 12 hr groups were given vitamin $K_1$ (●, 0.25 mg/100 g) or epoxide (○, 0.25 mg/100 g) intramuscularly or served as controls (△). Blood samples were taken at zero time and 5 and 10 hr after administration of vitamin $K_1$ or phylloquinone epoxide. Each point is the mean for three to five rats, with the vertical lines indicating the standard error.

B. Reversal by vitamin $K_1$ and phylloquinone epoxide of inhibition by tetrachloropyridinol (TCP). Rats that had received tetrachloropyridinol (6 mg/100 g) intraperitoneally 24 hr previously were treated with vitamin $K_1$ (●, 0.12 mg/100 g) or phylloquinone epoxide (○, 0.12 mg/100 g) intramuscularly or served as controls (△). Blood samples were taken at zero time and 5, 10, and 20 hr after administration of $K_1$ or epoxide. Each point is the mean for three rats, with the vertical lines indicating the standard error.

C. Reversal by phylloquinone epoxide of inhibition by 2-chloro-vitamin $K_1$ (Cl-K). Rats that had received 2-chloro-vitamin $K_1$ (0.3 mg/100 g) intracardially 18 hr previously were treated intramuscularly with phylloquinone epoxide (○, 0.12 mg/100 g) or served as controls (△). Blood samples were taken 5 and 10 hr after epoxide administration. Each point is the mean for three to six rats, with the vertical lines indicating the standard error.

2.5% (SE) of normal when fed Purina chow (11). Sixteen hours after the administration of vitamin, Sprague-Dawley and resistant rats were given an injection of warfarin, phenylindanedione, 2-chloro-vitamin $K_1$, or tetrachloropyridinol, and blood samples were taken 8 hr later (Tables 1 and 2). Plasma prothrombin was not decreased in the resistant rats treated with warfarin or phenylindanedione, whereas in Sprague-Dawley rats carried through the same experimental procedure, prothrombin decreased with a calculated half-life of 8–9 hr (Table 1). In contrast, 2-chloro-vitamin $K_1$ (0.3 mg/100 g) and tetrachloropyridinol (6 mg/100 g) appeared to block prothrombin synthesis completely in resistant rats, since the calculated half-life of prothrombin was 6–7 hr (Table 2). Even a dose of 2-chloro-vitamin $K_1$ as low as 0.05 mg/100 g blocked prothrombin synthesis effectively in resistant rats, while the same dose in Sprague-Dawley rats was not quite as effective. Similarly, tetrachloropyridinol at doses of 6.0 and 1.2 mg/100 g was slightly more effective in resistant than in Sprague-Dawley animals.

*Effect of phenylindanedione on response to phylloquinone epoxide in resistant and Sprague-Dawley rats.* Phylloquinone epoxide stimulates prothrombin synthesis in resistant rats given sufficient warfarin to block prothrombin synthesis completely in Sprague-Dawley animals, because the ability of the anticoagulant to block the conversion of epoxide to

TABLE 1

*Resistance of warfarin-resistant rats to phenylindanedione*

Rats received vitamin $K_1$ (25 μg/100 g) intramuscularly 16 hr before the intraperitoneal injection of phenylindanedione or warfarin. Blood samples were taken when the anticoagulants were administered (zero time) and 8 hr later.

| Rats | Dose | No. of rats | Prothrombin | |
|---|---|---|---|---|
| | | | 0 time | 8 hr |
| | *mg/100 g* | | *% control* | |
| Resistant | | | | |
| Warfarin | 0.3 | 16 | 108 | 120 |
| Phenylindanedione | 1.0 | 3 | 108 | 109 |
| Sprague-Dawley | | | | |
| Warfarin | 0.3 | 3 | 100 | 50 |
| Phenylindanedione | 1.0 | 3 | 114 | 64 |

TABLE 2

*Lack of resistance of warfarin-resistant rats to 2-chloro-vitamin $K_1$ and tetrachloropyridinol*

Rats received vitamin $K_1$ intramuscularly (25 μg/100 g) 16 hr before the intracardiac injection of 2-chloro-vitamin $K_1$ or intraperitoneal administration of tetrachloropyridinol. Blood samples were taken when the anticoagulants were administered (zero time) and 8 hr later. The results are the averages and standard errors for three to five rats.

| | Dose | Prothrombin | |
|---|---|---|---|
| | | 0 time | 8 hr |
| | *mg/100 g* | *% control* | |
| Resistant | | | |
| 2-Chloro-vitamin $K_1$ | 0.3 | 108 | 45 ± 10 |
| | 0.05 | 108 | 57 ± 8 |
| Tetrachloropyridinol | 6.0 | 108 | 50 ± 5 |
| | 1.2 | 108 | 70 ± 7 |
| Sprague-Dawley | | | |
| 2-Chloro-vitamin $K_1$ | 0.05 | 100 | 70 ± 8 |
| Tetrachloropyridinol | 6.0 | 100 | 63 ± 10 |
| | 1.2 | 100 | 82 ± 12 |

vitamin $K_1$ is greatly reduced in the resistant strain (10, 11). Phylloquinone epoxide also stimulated prothrombin synthesis in resistant rats given phenylindanedione 0.5 hr previously (Fig. 4). For controls Sprague-Dawley rats, which had low prothrombin levels from eating a vitamin K-deficient diet, were treated in the same way. The response to the epoxide was completely blocked by phenylindanedione in these animals.

*Vitamin K antagonists and metabolism of [$^3H$]vitamin $K_1$.* Since warfarin has such a marked effect on the metabolism of vitamin K, the other antagonists were studied. Previously it was found that warfarin increased the hepatic ratio of labeled phylloquinone epoxide to vitamin $K_1$ 9-fold compared with controls after injection of 100 μg of [$^3$H]-vitamin $K_1$ (2). After administration of a tracer dose of [$^3$H]vitamin $K_1$ (5 ng/100 g), warfarin also increased the epoxide to $K_1$ ratio 6–19-fold, compared with controls, at 2 and 5 hr (Table 3). Although warfarin had little effect on the total $^3$H in the liver, the amount of [$^3$H]vitamin $K_1$ was markedly reduced in comparison with controls, presumably because it was trapped as the epoxide. Phenylindanedione also increased the amount of tritiated epoxide relative to vitamin $K_1$ by 6–7-fold, and decreased the hepatic level of [$^3$H]vitamin $K_1$ as compared to controls 2 and 5 hr after administration of labeled vitamin. In contrast, in the rats treated with 2-chloro-vitamin $K_1$ and tetrachloropyridinol the relative amount of [$^3$H]phylloquinone epoxide was only slightly greater than in the controls. Also, the hepatic level of [$^3$H] vitamin $K_1$ was not much different from that found in the controls.

*Vitamin K antagonists and metabolism of [$^3H$]phylloquinone epoxide.* A nearly physiological dose (170 ng/100 g) of [$^3$H]phylloquinone epoxide was readily converted to [$^3$H]vitamin $K_1$ (Table 4). The epoxide to $K_1$ ratio 2 hr after administration was close to that obtained when the tritiated vitamin was administered, indicating that the interconversion between $K_1$ and epoxide was near equilibrium. Warfarin and phenylindanedione blocked the epoxide to $K_1$ conversion and increased the amount of epoxide as compared to controls about equally well. The

epoxide to vitamin $K_1$ ratios were increased about 18-fold over the controls, and the amount of hepatic [$^3$H]phylloquinone epoxide was also significantly increased. The other anticoagulants tetrachloropyridinol and 2-chloro-vitamin $K_1$, also appeared to inhibit the epoxide to $K_1$ conversion but not nearly as effectively. 2-Chloro-vitamin $K_1$ also increased the amount of [$^3$H]phylloquinone epoxide as compared to controls, but tetrachloropyridinol had no significant effect. The hepatic [$^3$H]epoxide:[$^3$H]$K_1$ ratio was 0.96 at 2.5 hr after administration of 2-chloro-vitamin $K_1$, suggesting that the epoxide would be ineffective against the anticoagulant shortly after 2-chloro-vitamin $K_1$ administration. However, phylloquinone epoxide was fully effective in overcoming inhibition of prothrombin synthesis 18 hr after injection of 2-chloro-vitamin $K_1$ (Fig. 3C).

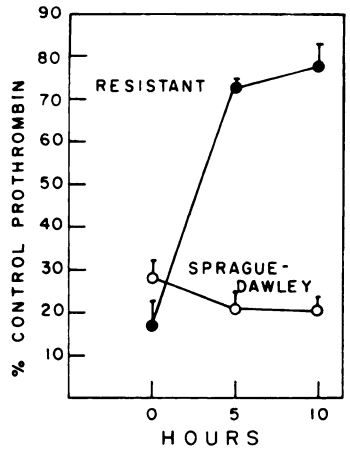


FIG. 4. *Response to phylloquinone epoxide by Sprague-Dawley and warfarin-resistant rats treated with phenylindanedione*

Sprague-Dawley rats fed vitamin K-deficient diet for 12 days (○) and resistant rats fed Purina rat chow (●) received phenylindanedione (1 mg/100 g) intraperitoneally 0.5 hr before the intramuscular injection of phylloquinone epoxide (0.12 mg/100 g). Each point is the mean for three or four rats, with the vertical lines indicatng the standard error.

## DISCUSSION

The antagonists of vitamin K were all found to block prothrombin synthesis completely, without a substantial lag. Experiments to determine whether the mechanisms of action of the antagonists were similar indicated that they could be divided into two groups. The inhibition of prothrombin synthesis by all four anticoagulants was reversed by vitamin $K_1$, but the inhibition in Sprague-Dawley rats by group I antagonists (warfarin and phenylindanedione) was not reversed by phylloquinone epoxide. However, the epoxide did cause a slight increase in plasma prothrombin in both warfarin-

TABLE 3

*Antagonists and metabolism of [$^3$H]vitamins $K_1$*

Rats received an intracardiac injection of 5 ng/100 g of [$^3$H]vitamin $K_1$, and their livers were removed and analyzed as previously described (1, 11) 2 and 5 hr later. Groups were given an intraperitoneal injection of warfarin (0.1 mg/100 g), phenylindanedione (1 mg/100 g), or tetrachloropyridinol (6 mg/100 g), or an intracardiac injection of 2-chloro-vitamin $K_1$ (0.3 mg/100 g), 0.5 hr before injection of the radioactive vitamin. The standard errors of the results are shown.

| Treatment | 2 hr | | | | 5 hr | | | |
|---|---|---|---|---|---|---|---|---|
| | No. of rats | Liver | [$^3$H]$K_1$ | [$^3$H]Epoxide: [$^3$H]$K_1$ | No. of rats | Liver | [$^3$H]$K_1$ | [$^3$H]Epoxide: [$^3$H]$K_1$ |
| | | *% injected $^3$H* | | | | *% injected $^3$H* | | |
| Control | 10 | 26 | 8.6 ± 0.9 | 0.16 ± 0.03 | 5 | 10 | 4.0 ± 1.1 | 0.25 ± 0.07 |
| Warfarin | 5 | 31 | 3.1 ± 0.3 | 3.0 ± 0.2 | 6 | 15 | 1.4 ± 0.2 | 1.6 ± 0.3 |
| Phenylindanedione | 3 | 34 | 4.1 ± 0.8 | 1.1 ± 0.1 | 3 | 12 | 1.0 ± 0.0 | 1.4 ± 0.5 |
| 2-Chloro-vitamin $K_1$ | 5 | 23 | 6.0 ± 0.4 | 0.34 ± 0.05 | | | | |
| Tetrachloropyridinol | 4 | 23 | 10.4 ± 2.9 | 0.26 ± 0.02 | | | | |

TABLE 4

*Antagonists and metabolism of [$^3H$]phylloquinone epoxide*

Rats were given an intracardiac injection of 170 ng/100 g of [$^3$H]phylloquinone epoxide, and their livers were removed and analyzed as previously described (1, 11) 2 hr later. Groups received warfarin (0.1 mg/100 g), phenylindanedione (1 mg/100 g), or tetrachloropyridinol (6 mg/100 g) intraperitoneally or 2-chloro-vitamin $K_1$ intracardially (0.3 mg/100 g) 0.5 hr before injection of the radioactive epoxide. The standard errors of results are shown.

| Treatment | No. of rats | Liver | [$^3$H]Epoxide | [$^3$H]Epoxide:[$^3$H]$K_1$ |
|---|---|---|---|---|
| | | *% injected $^3H$* | | |
| Control | 4 | 41 | 4.3 ± 0.7 | 0.24 ± 0.01 |
| Warfarin | 2 | 36 | 12.6 ± 1.6 | 3.8 |
| Phenylindanedione | 4 | 38 | 16.5 ± 2.8 | 4.6 ± 1.3 |
| 2-Chloro-vitamin $K_1$ | 6 | 41 | 10.2 ± 0.8 | 0.96 ± 0.23 |
| Tetrachloropyridinol | 3 | 30 | 5.5 ± 0.1 | 0.51 ± 0.10 |

and phenylindanedione-treated rats (Fig. 3) (15). The reason for this is not clear, although possibly phylloquinone epoxide has some vitamin K activity in addition to being an inhibitor of the vitamin. Metabolic experiments with [$^3$H]phylloquinone epoxide demonstrated that group I compounds inhibited the conversion of epoxide to active vitamin $K_1$ (Table 4). Group I also caused the accumulation of labeled epoxide in rats given tritiated vitamin $K_1$. It has been proposed that group I antagonists are ineffective in blocking prothrombin synthesis in warfarin-resistant rats because in these animals the epoxide to $K_1$ conversion is not as sensitive to these antagonists as in normal animals (11, 16). Consequently phylloquinone epoxide can stimulate prothrombin synthesis in the presence of group I compounds in the resistant strain (Fig. 3) (11). In contrast, the inhibition of prothrombin synthesis by group II compounds (tetrachloropyridinol and 2-chloro-vitamin $K_1$) was reversed by epoxide, because they inhibited the epoxide to vitamin $K_1$ conversion only slightly compared to group I. Similarly, the effect of group II on the metabolism of [$^3$H]vitamin $K_1$ was much less than that of group I. Group II blocked prothrombin synthesis in warfarin-resistant animals at least as well as in Sprague-Dawley rats. These results are consistent with the idea that the coumarin and indanedione anticoagulants (group I) inhibit prothrombin synthesis by causing the accumulation of phylloquinone epoxide, an inhibitor of the vitamin. In resistant animals warfarin and phenylindanedione do not inhibit the conversion of the epoxide back to vitamin $K_1$, and the epoxide does not accumulate (11, 16). 2-Chloro-vitamin $K_1$ and tetrachloropyridinol do not appear to block prothrombin synthesis by the same mechanism as phenylindanedione and warfarin. 2-Chloro-vitamin $K_1$ could compete with the vitamin at the active site because of structural similarity, as Lowenthal has pointed out (3), but tetrachloropyridinol has no obvious resemblance to vitamin K. Preliminary evidence indicates that 2-chloro-vitamin $K_1$ and tetrachloropyridinol inhibit the epoxidation of vitamin $K_1$ *in vivo* and *in vitro*.[1] This reaction may be involved in the methanism of action of vitamin K, since Willingham and Matschiner (17) found that liver vitamin $K_1$ epoxidase activity was inversely proportional to plasma prothrombin concentration.

Thierry and Suttie (18) found that 2-chloro-vitamin $K_1$ caused a 2–3-fold increase in hepatic radioactivity after injection of [$^{14}$C]vitamin $K_1$, with most of the increase in the mitochondrial fraction. We found that the chloro analogue had little effect on the amount of $^3$H in the liver after administration of [$^3$H]vitamin $K_1$ or [$^3$H]phylloquinone epoxide (Tables 3 and 4). Possible differences in results might be due to the simultaneous administration of 2-chloro-vitamin $K_1$ and [$^{14}$C]vitamin $K_1$ or the much larger dose of labeled vitamin used in the former study (5 μg/100 g). We injected 5 ng/100 g of [$^3$H]-

[1] A. K. Willingham, R. L. Laliberte, and R. G. Bell, manuscript in preparation.

vitamin $K_1$, which is close to a tracer dose, since a 10-g rat liver contains approximately 700 ng of vitamin K (19). We found that 2-chloro-vitamin $K_1$ lowered slightly the amount of unmetabolized vitamin $K_1$ in the liver, apparently by increasing the relative amount of phylloquinone epoxide (Table 3). Consistent with this was the moderate inhibition by 2-chloro-vitamin $K_1$ of the conversion of [$^3$H]phylloquinone epoxide to [$^3$H]vitamin $K_1$ (Table 4).

The ability of tetrachloropyridinol and 2-chloro-vitamin $K_1$ to block prothrombin synthesis at least as effectively in warfarin-resistant as in Sprague-Dawley animals suggests that these anticoagulants may be useful as rodenticides in areas where there are large numbers of rats genetically resistant to coumarin and indanedione rodenticides (20, 21). Shah and Suttie (22, 23) have recently found that the chloro analogue was more effective in resistant than in Holtzman rats and that it could be used to kill resistant rats when fed in the diet. Martin (24) also reported that 2-chloro-vitamin $K_1$ was as effective in warfarin-resistant as in normal rats but that coumatetralyl, a coumarin, and chlorphacinone, an indanedione, were much less effective in resistant animals.

ACKNOWLEDGMENTS

The authors wish to acknowledge the invaluable technical assistance of Ms. Deborah Chisholm, Ms. Laurel Truesdell, and Mr. Paul Caldwell.

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