

## Vitamin K Requirement and Warfarin Tolerance in the Hamster<sup>1</sup> (38987)

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The Syrian hamster has been reported by Hamilton and Hogan (1) to have a dietary requirement for vitamin K. Granados (2) failed to demonstrate such a requirement, but it is likely that differences in intestinal flora may have caused varying results in these two studies. During our recent studies on plasma abnormal prothrombin and liver prothrombin precursor activity in different species (3), we observed that hamsters developed hypoprothrombinemia when administered the anticoagulant, 2-chloro-3-phytyl-1,4-naphthoquinone (chloro-K) but did not respond to the anticoagulant Warfarin. In this regard they were similar to the strains of wild rats that have been found by various investigators (4-7) to be resistant to Warfarin and other coumarin anticoagulants. We have previously reported (8, 9) that the Warfarin-resistant rat has about a 20-fold higher requirement for vitamin K<sub>1</sub> than does the normal rat. Because of the apparent similarity of the hamster to the Warfarin-resistant rat, and the lack of conclusive data on the requirement of vitamin K in this species, the response of the hamster to anticoagulants and vitamin K was studied in more detail.

*Methods.* Male, 125-145 g hamsters (A. R. Schmidt, Madison, WI) were used throughout these studies. Hypoprothrombinemia was produced either by feeding a vitamin K-deficient diet (10) to animals housed in coprophagy preventive cages (11); or by treating them with the vitamin K antagonists Warfarin or 2-chloro-3-phytyl-1,4-naphthoquinone (chloro-K). Sodium Warfarin was administered intraperitoneally in 0.9% NaCl

and chloro-K was emulsified in Tween 80 in 0.9% NaCl for intracardial injections.

Blood was drawn from 18-hr fasted hamsters by cardiac puncture into a syringe containing 0.15 M potassium oxalate (10% final volume). The blood was centrifuged at 2000g for 20 min in a refrigerated centrifuge. Plasma prothrombin concentrations were measured by the two-stage method of Ware and Seegers as modified by Shapiro and Waugh (12) and expressed as NIH units per ml of plasma. Normal hamsters were found to have prothrombin concentrations ranging from 200 to 230 units with an average of 222 U/ml. Prothrombin concentrations following anticoagulant treatment or nutritional deficiency were expressed as a percentage of this normal average value.

*Results.* Plasma prothrombin levels following treatment with chloro-K (1-5 mg/kg body wt) were found to decline to 17-20% of average control values. Similar treatment with Warfarin had no effect on plasma prothrombin concentrations (Table I). Increasing the Warfarin dose to 25 mg/kg had a slight effect on plasma prothrombin, and a dose of 100 mg/kg appeared to almost completely block prothrombin synthesis. These observations on the response of plasma prothrombin levels to Warfarin administration suggested that the hamsters might be similar to the Warfarin-resistant rat and have a high requirement for vitamin K.

Male hamsters (125-145 g) were housed in coprophagy preventive cages and fed a vitamin K-deficient rat diet. Plasma prothrombin concentrations were reduced as shown in Table II. The cages used during these studies were the same that were routinely used for 200-220 g rats, and it is possible that some coprophagy may have occurred.

An intramuscular injection of phylloqui-

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TABLE I. EFFECT OF ANTICOAGULANTS ON PLASMA PROTHROMBIN CONCENTRATIONS OF HAMSTERS.<sup>a</sup>

Anti-coagulant treatment (mg/kg body wt)	Prothrombin concentration (% of control)	
	Warfarin (intraperitoneal)	Chloro-K (intracardial)
1.0	103.08 ± 3.98 (6) <sup>b</sup>	17.45 ± 3.21 (5)
2.5	—	17.71 ± 1.48 (5)
5.0	102.42 ± 3.70 (5)	19.26 ± 0.88 (14)
10.0	—	16.46 ± 2.49 (4)
25.0	75.57 ± 5.07 (6)	—
100.0	22.15 ± 1.08 (3)	—

<sup>a</sup> Anticoagulants were injected 24 hr before the experiments, and the blood was drawn by heart puncture.

<sup>b</sup> Values are mean ± SE (number of animals).

TABLE II. DEVELOPMENT OF A DIETARY DEFICIENCY OF VITAMIN K.<sup>a</sup>

Days on diet	Plasma prothrombin (% of control)
6	37.59 ± 4.70 (13) <sup>b</sup>
9	25.44 ± 4.73 (4)
11	10.69 ± 4.39 (5)

<sup>a</sup> Male hamsters were fed a vitamin K-deficient diet and housed in coprophagy preventing cages for the periods indicated.

<sup>b</sup> Values are mean ± SE (number of animals).

none (5 mg/kg) to vitamin K-deficient hypoprothrombinemic normal rats will completely reverse the deficiency and restore about half of the normal circulating levels of prothrombin in 1 hr. A similar dose (5 mg/kg) of vitamin K<sub>1</sub> to vitamin K-deficient hypoprothrombinemic male hamsters showed very poor response at the first hr. When the intramuscular dose of vitamin K<sub>1</sub> administered was doubled (10 mg/kg), about half of the steady-state plasma levels of prothrombin were restored within 60–90 min. A similar dose of vitamin K<sub>1</sub> (10 mg/kg) to chloro-K pretreated (1–5 mg/kg) hamsters restored about 20–40% of the normal steady-state levels at first hr after vitamin K injection. These results indicate that hypoprothrombinemic hamsters have higher requirements for vitamin K<sub>1</sub> than does the rat.

*Discussion.* These data clearly demonstrate that it is possible to produce a nutritional deficiency of vitamin K in the hamster and suggest that the disagreement in earlier reports (1, 2) may have been caused by varying amounts of intestinal synthesis of the vitamin, or varying amounts of coprophagy practiced by the animals in the two different studies. The data obtained also indicate that the hamster is more sensitive to chloro-K, a direct antagonist of vitamin K, and much more resistant to the action of Warfarin than is the common laboratory rat. The normal rat requires less than 1 mg/kg Warfarin and over 5 mg/kg chloro-K to completely block prothrombin synthesis. The hamster is therefore much more like the Warfarin-resistant rat (9) than the common laboratory rat. The large amount of vitamin needed to overcome the hypoprothrombinemia produced by chloro-K administration would also suggest that, in common with the resistant rat, the hamster has a high vitamin K requirement. Various theories have been advanced (8, 13, 14) to explain the Warfarin resistance, and studies in hamsters might contribute to an understanding of this interesting genetic alteration.

*Summary.* A dietary deficiency of vitamin K has been demonstrated in the Syrian hamster. This species has also been shown to be relatively resistant to the action of the indirect anticoagulant Warfarin, and very sensitive to the anticoagulant action of the vitamin K antagonist chloro-K. These observations, and the hamster's apparently high requirement for the vitamin, indicate that it responds to vitamin K and vitamin K antagonists in the same fashion as Warfarin-resistant strains of rats.

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