Effect of Sex and Sex Hormones on Plasma Prothrombin and Vitamin K Deficiency[1] (37581)

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The interaction of vitamin K and sex hormones in regulating the concentrations of clotting proteins is of interest because of the insight it may give into the mechanism of action of the vitamin and because of the possible connection with thromboembolic disease—particularly in pregnant women and those taking oral contraceptives. It has been observed that in humans, pregnancy (1, 2) and oral contraception (3–6) cause elevation of the vitamin K dependent clotting proteins in plasma. The rat appears to be a useful animal to study interactions between sex hormones, vitamin K and clotting proteins because the prothrombin levels and susceptibility to vitamin K deficiency are clearly influenced in these animals (7–10). Estradiol protects against hypoprothrombinemia while testosterone enhances the lowering of plasma prothrombin in rats fed vitamin K-deficient diets (8, 9).

As part of our continued interest in vitamin K and its effects in animal systems, we have undertaken a study of the effect of sex and sex hormones on the synthesis of clotting proteins. The first results of the study are presented in this report.

Materials and Methods. Sprague–Dawley rats were obtained from Charles River and Carworth Laboratories. Prothrombin was assayed according to Hjort, Rapaport and Owren (11, 12). Control plasma was pooled from 10–11-wk-old male rats. Vitamin K deficiency was induced in rats as described by Matchiner and Taggart (13). The synthetic vitamin K-deficient diet was prepared by General Biochemicals.

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Solutions of vitamin K_1 and estradiol for injection were prepared by dissolving the lipids in Tween 80 and diluting the solution with 0.9% NaCl to make a solution containing 5–10% Tween.

Results. Plasma prothrombin in the rat. Circulating plasma prothrombin in the rat has been reported to differ with the sex of the animal. Plasma prothrombin was measured in male and female rats to investigate the sex difference and to determine if there was an increase in prothrombin in females around puberty when estrogen would be secreted. Prothrombin concentration in both sexes increased with age but the level was higher in females up to 19 wk. The greatest increase in plasma prothrombin in female rats occurred between 4.5 and 6.5 wk of age which may coincide with puberty and the release of estrogen but the greatest increase in prothrombin in males also occurred during this time (Fig. 1).

To ascertain if the low level of prothrombin found in young rats was due to vitamin K deficiency, 4-wk-old male rats fed Purina Chow were injected with 1 mg of vitamin K_1 intramuscularly. There was no significant increase in plasma prothrombin at 24 hr after injection of the vitamin.

Plasma prothrombin in pregnant rats. Since the levels of the prothrombin complex clotting factors are elevated during pregnancy in humans (1, 2), we determined prothrombin concentration during pregnancy in rats (Table I). Prothrombin increased steadily throughout pregnancy to a maximum 140% of control at 3 days before delivery. The prothrombin level was decreased 2 days postpartum approximately to the level

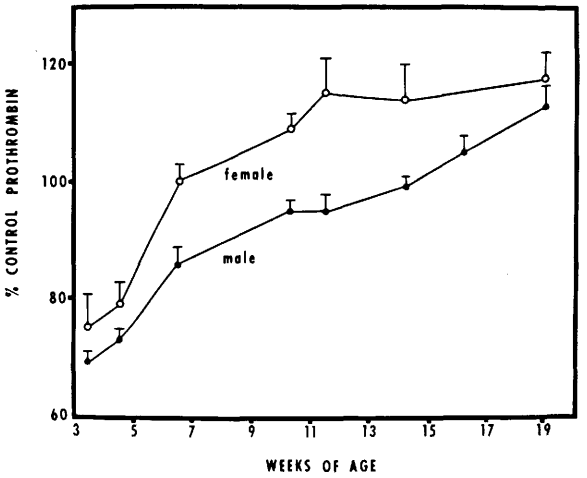

FIG. 1. Plasma prothrombin in rats. Male (●) and female (○) rats were fed Purina Laboratory Chow. Blood samples were taken at the ages shown. Each point is the average of 5–15 rats with the standard error of the mean.

found in rats of the same age (13 wk) which are nonpregnant (Fig. 1).

Turnover of prothrombin in pregnant rats. To determine if the elevated prothrombin levels in pregnant rats were due to a decreased rate of turnover of plasma prothrombin, the half-life of the clotting protein was measured after blocking further prothrombin synthesis with warfarin (Fig. 2). Prothrombin in pregnant rats decreased logarithmically with a half-life of 7–8 hr which is not significantly different than the half-life of plasma prothrombin in male rats [Fig. 2 and Ref. (14)].

Induction of vitamin K deficiency in male and female rats. To confirm previous reports of sex differences in the development of vitamin K deficiency (7–10), male and female rats were fed a synthetic deficient diet (Table II). Prothrombin levels in males decreased

TABLE I. Prothrombin in Pregnant Rats.[a]

Days after conception	Prothrombin[b] (% of control)
0	109 ± 4
4	112 ± 2
9	127 ± 3
14	136 ± 4
18	140 ± 8
Postpartum (2 days)	120 ± 6

[a] Timed pregnant Sprague–Dawley rats which were 10 wk old at conception were fed Purina Rat Chow.

[b] Each value is the average of 7 rats ± the standard error of the mean. The control plasma was from 10–11 wk old male rats.

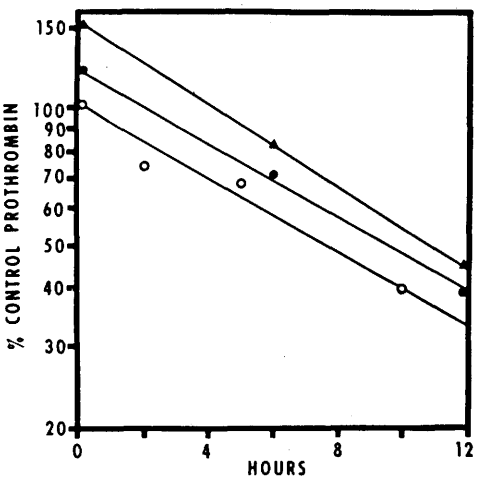

FIG. 2. Half-life of prothrombin in pregnant rats. Two rats on Day 19 of pregnancy were injected intraperitoneally with warfarin (1 mg/100 g body wt). Initial prothrombin concentrations were 152 (▲) and 120 (●) % of control. For comparison 3 male rats with initial prothrombin concentration 100% of control (○) were injected with the same dose of warfarin. The half-lives of prothrombin in the pregnant rats estimated from these data were 6.9 and 7.5 hr compared with 7.6 hr in the males.

TABLE II. Induction of Vitamin K Deficiency in Male and Female Rats.[a]

	Prothrombin concn[b] (% of control)		
	11 days	28 days	35 days
Male	18 ± 2 (10)		
Female	111 ± 7 (9)	51 ± 4 (12)	53 ± 8 (10)

[a] Male and female rats (10–11 wk old) were fed a vitamin K-deficient diet for the indicated length of time.

[b] Prothrombin concentration ± standard error. In parentheses is the number of rats in each group.

rapidly while the deficiency remained moderate in females even after 5 wk of feeding the deficient diet.

Influence of estrogen on vitamin K-deficient male rats. To test whether the sex difference was due to stimulation of prothrombin synthesis by estrogen, vitamin K-deficient male rats were treated with estradiol or Premarin (Ayerst Laboratories), a mixture of conjugated estrogens. No response was observed for up to 24 hr in either adult or immature males (Table III). Since it had been reported that as little as 20 μg of estradiol would restore normal prothrombin levels in deficient rats within 6 hr (15) we administered estradiol using a number of different solvents and injection routes but no response was observed during a period of 24 hr following injection of the estrogen. These results indicate that the influence of estrogen on vitamin K-deficient male rats occurs more slowly than previously recognized. This is supported by the results in Fig. 3 which show a gradual rise in plasma prothrombin in deficient adult male rats over several days following the injection of estradiol.

Discussion. There appear to be two distinct effects of sex on the level of vitamin K dependent clotting proteins in the rat: (a) the concentration of these proteins is higher in females and, (b) the dietary requirement for vitamin K to maintain normal concentrations of clotting proteins in females is lower. In both rats and man, pregnancy appears to release an upper control mechanism resulting in levels of clotting protein higher than observed in normal females [Table I and Refs. (1, 2, 6)]. In rats, females have higher prothrombin levels than males (Fig. 1) but more limited studies suggest that this is not the case in humans.

Elevation of the concentration of a protein may result from an increase in the rate of synthesis or a decrease in the rate of turnover or both. The half-life of prothrombin in pregnant rats was not significantly different from that found in male rats indicating that elevated prothrombin levels in pregnancy are due to an increased rate of appearance of the clotting factor in plasma. Thus the first effect of sex, elevation of clotting protein, appears to be due to an increase in the rate of

TABLE III. Plasma Prothrombin in Vitamin K-Deficient Male Rats Treated with Estrogen or Vitamin K_1.

Treatment[a]	Mode of administration[b]	Prothrombin concn[c] (% of control) 0	2 hr	8 hr	24 hr
Mature rats					
0.1 mg estradiol in 50% ethanol	ic	24	13		
0.1 mg estradiol in Tween emulsion	ic	24			23
0.5 mg estradiol in sesame oil	sc	31			21
1 mg estradiol in Tween emulsion	im	13			9
1 mg of Premarin in 0.9% NaCl	ip	21	15		14
0.2 mg vitamin K_1 in Tween emulsion	ic	25	55	85	100
Immature rats					
0.1 mg estradiol in Tween emulsion	ic	23			16
0.5 mg estradiol in Tween emulsion	im	19		22	26
0.5 mg vitamin K_1 in Tween emulsion	im	21		69	102

[a] Mature (10–15 wk) and immature (30 days) male rats were fed vitamin K deficient diet for 10–14 days prior to the treatment.

[b] ic, intracardial; sc, subcutaneously; im, intramuscularly; ip, intraperitoneally.

[c] Prothrombin concentration is shown for varying times up to 24 hr following administration of estrogen or vitamin K_1. Each value is the average for 3 or more rats.

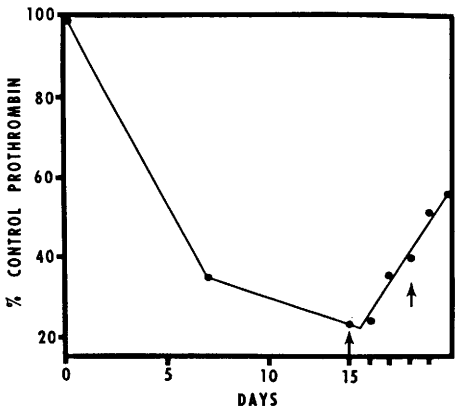

FIG. 3. Influence of estradiol on prothrombin in vitamin K-deficient male rats. Ten 19-wk-old male rats were fed vitamin K-deficient diet throughout the experiment. The arrows indicate the injection of 1 mg of estradiol valerate subcutaneously.

formation of the protein. Whether the second effect, the decrease in vitamin K requirement, is related to the first cannot be determined with present evidence.

The data in Table III appear to rule out the possibility that estradiol has the activity of vitamin K or otherwise directly stimulates the synthesis of prothrombin. The concentration of plasma prothrombin increased rapidly in deficient male rats following the injection of vitamin K; but in similar experiments no increase in prothrombin was observed for 24 hr following the administration of estrogen. The time required for the effect of estrogen on vitamin K deficiency may be estimated from the data in Fig. 3 which show a slow rise in prothrombin in deficient male rats over 5 days following an initial subcutaneous injection of estradiol valerate. Explanations for the effect of administered estrogen in longer-term experiments are: (a) that the absorption of vitamin K in the gut is made more efficient, (b) that the turnover of vitamin K in liver is decreased, and (c) that liver, in the formation of prothrombin, becomes more responsive to lower concentrations of vitamin K. It should be apparent that the decreased requirement for vitamin K and the increased circulating levels of prothrombin in female or estrogen-treated rats may be due to separate effects of the hormone. Thus more than one of the above possibilities may be operative.

The results reported here indicate that the concentration of plasma prothrombin in the rat increases gradually in both sexes with age and that the sharpest increase occurs during puberty. Except for the oldest animals studied, the concentration of plasma prothrombin was consistently higher in the female than in the male. A brief report of a similar study by Mellette (9) indicated a gradual decline in prothrombin concentration in male rats with age, but the diet used in these studies may have been deficient in vitamin K. The results of the present study indicate that sexual maturity in both sexes is required for maximum synthesis of prothrombin, and that the rate of formation of prothrombin is highest in the female.

Summary. In rats as in man, pregnancy results in levels of prothrombin higher than observed in normal females. In the rat, prothrombin levels are higher in the female than in the male and the dietary requirement for vitamin K in the female is less. Higher levels of plasma prothrombin in the female are not due to decreased turnover of the protein. Estradiol does not have the activity of vitamin K or otherwise directly stimulate the formation of prothrombin. The effect of estradiol on vitamin K deficiency in male rats occurs slowly over a period of several days. The decreased requirement for vitamin K and the increased circulating levels of prothrombin in female or estrogen-treated rats may be due to separate effects of the hormone.

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