EFEECT OF VITAMIN K_3 AND TESTOSTERONE PROPIONATE ON PLASMA CHOLESTEROL LEVEL AND PROTHROMBIN RATE IN CASTRATED AND INTACT RATS

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Summary: Adult castrated and intact rats of the Charles River strain were used to determine the effect of various dose levels of vitamin K_3 and testosterone propionate on plasma prothrombin rate and cholesterol level. Vitamin K_3 at the level of 0.1 mcg per g of diet was adequate in maintaining normal prothrombin rate in the castrated rats but not in the intact rats. However, vitamin K_3 at the rate of 0.5 mcg per g of diet maintained normal prothrombin rate in both the castrated and intact rats. The effect of castration or vitamin K_3 was to maintain prothrombin rate towards normal. Interactions of castration and vitamin K_3, castration and testosterone, and of vitamin K_3 and testosterone on prothrombin rate were significant in the third experiment. The initial plasma cholesterol level of the castrated rats was significantly higher compared to the intact rats. The hypocholesteremic effect of 0.1 mcg vitamin K_3 per g of diet was significant only in the castrated rats. Vitamin K_3 at a dose level of 1.0 mcg per g of diet had a hypocholesteremic effect both in the castrated and intact rats.

The results indicate that castrated rats have a lower vitamin K_3 requirement than intact rats and that vitamin K_3 appears to be involved not only in prothrombin synthesis but also in cholesterol metabolism.

Key Words: *vitamin K_3 testosterone propionate plasma cholesterol prothrombin rate effect of castration.*

INTRODUCTION

We have previously observed (13) that the serum cholesterol level of rats rose markedly when they were shifted from a commercial vitamin K-adequate rat diet to a synthetic vitamin K-free diet. No information is available regarding the effect of vitamin K on cholesterol metabolism in rats. The observation that serum cholesterol increased after rats were fed a vitamin K-free diet indicated the possible hypocholesteremic effect of vitamin K in this species. The role of vitamin K in blood coagulation is well established and sex hormones appear to regulate vitamin K requirements. Hougie *et al* (2) reported that estrogens had a vitamin K-like effect

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on blood coagulation in women. Male rats were found to be more susceptible to hemorrhagic diathesis than female rats (4, 9, 10) and intact rats were more susceptible to hemorrhages than castrated rats when fed irradiated beef diet (5, 6). Testosterone was reported to significantly decrease and vitamin K_3 significantly increase the prothrombin rate of rats fed irradiated beef(6).

Three types of experiments were designed to investigate the role of vitamin K_3 and testosterone propionate on blood cholesterol level both in the intact and castrated rats. Since the effect of vitamin K on prothrombin rate is well established, it was designed to check the adequacy or deficiency of the dose levels of vitamin K_3 used and also to determine if the vitamin K requirements for the intact and the castrated rats were different.

MATERIALS AND METHODS

Three experiments were conducted using 18-20 week old rats of Charles River strain. Rats were castrated two weeks prior to the start of the experiment. Intact rats weighed about 480 *g* and the castrated rats weighed about 450 *g*. In all experiments rats were divided into experimental groups on the basis of initial plasma-cholesterol values. In the first experiment, 22 intact and 22 castrated rats were used for an experimental period of 36 days. The intact and the castrated rats were each divided into two groups (intact groups 1 and 2 and castrated groups 3 and 4). Rats of groups 1 and 3 were fed the basal vitamin K-deficient diet of Mameesh and Johnson (7) except that USP salt mix XVI was used. Rats of groups 2 and 4 were fed the basal diet plus 0.1 *mcg* of vitamin K_3 (menadione) per g of diet. All rats were bled at the beginning and after 18 and 36 days on experimental diets for plasma cholesterol and prothrombin time determination.

In the second experiment, three groups of 5 castrated rats each and a fourth group of 5 intact rats were used. Group 1 was fed the basal diet; group 2 was fed the basal diet plus 0.1 mcg of vitamin K_3 per *g* of diet; group 3 was fed the basal diet plus 0.5 *mcg* of vitamin K_3 per g of diet. The intact rats were fed the basal diet plus 0.5 *mcg* vitamin K_3 per *g* of diet. The bleeding schedule was the same as in the first experiment and both plasma cholesterol and plasma prothrombin time were determined.

The third experiment consisted of eight groups of 6 rats each. Groups 1, 2, 3 and 4 contained castrated rats, while intact rats were used for groups 5, 6, 7 and 8. Group 1 was fed the basal diet and injected subcutaneously with 0.2 *ml* sesame oil twice a week ; group 2 was fed the basal diet and injected subcutaneously with 5 *mg* testosterone propionate in 0.2 *ml* sesame oil twice a week; group 3 was fed the basal diet plus 1 *mcg* vitamin K_3 per *g* of diet and injected with sesame oil as in group 1; group 4 was fed the same diet as group 3 but injected with 5 *mg* testosterone propionate as in group 2. Rats of groups 5, 6, 7 and 8 were given the same treatment as rats of groups 1, 2, 3 and 4 respectively. The experimental period was 18 days and all were bled at the start and the end of the experiment. Plasma cholesterol and prothrombin time were determined.

In all experiments food and water was supplied ad libitum. Rats were weighed individually each week. A fibrometer coagulation timer was used for the prothrombin time (PT) determination according to the method of Quick (12). Plasma cholesterol was determined using Hycel cholesterol reagent (Hycel, Inc., Houston). It was thought that the in tial plasma cholesterol concentration might influence the final plasma cholesterol level and, to eliminate this possible effect, the plasma cholesterol changes rather than actual plasma cholesterol values were analyzed. These differences were calculated for each rat by subtracting its initial plasma cholesterol value from its final cholesterol value. Only those rats for which complete data were available throughout the experiment were included in statistical analysis. Plasma prothrombin times were transformed to prothrombin rates $\frac{1}{\text{PT (Sec.)}} \times 1000$ for statistical analysis. All data were analyzed by analysis of variance (14).

RESULTS AND DISCUSSION

Mean plasma prothrombin rate as well as mean initial plasma cholesterol level and mean changes in cholesterol level on experimental days 18 and 36 are shown in Table I. The initial prothrombin rates of all treatment groups were statistically equal. Although both castration and vitamin K_3 increased the prothrombin rate, on experimental day 18 the effect of only castration was statistically significant ($P<0.005$), that of vitamin K_3 and K_3 plus castration interaction did not reach statistical significance. However, with prolonged vitamin K depriva-

TABLE I: *Effect of vitamin K_3 on plasma cholesterol and plasma prothrombin rate of castrated and intact rats fed synthetic diet.*

Experimental Day	*mcg vitamin K_3 per g of diet*			
	0		0.1	
	Castrate	Intact	Castrate	Intact
	Plasma Cholesterol			
0	84.1 ± 4.67 (8)*	76.8 ± 4.71 (8)	86.2 ± 3.59 (11)	68.3 ± 4.90 (7)
18	+32.5 ± 3.62 (8)**	+22.9 ± 6.33 (8)	+13.3 ± 3.06 (11)	+29.0 ± 2.48 (7)
36	+29.5 ± 3.28 (8)	+26.1 ± 5.95 (8)	+21.6 ± 4.61 (11)	+24.6 ± 2.61 (7)
	Plasma Prothrombin Rate			
0	80.3 ± 2.93 (6)†	78.0 ± 1.84 (7)	79.1 ± 1.91 (9)	80.5 ± 1.53 (8)
18	75.0 ± 2.89 (6)	58.7 ± 7.37 (7)	82.3 ± 1.14 (9)	65.6 ± 3.20 (8)
36	64.3 ± 4.12 (6)	49.6 ± 7.20 (7)	80.8 ± 1.48 (9)	58.0 ± 3.23 (8)

*Mean plasma cholesterol (*mg* per 100 *ml* plasma) ± S.E. for number of rats in parentheses.

**Mean plasma cholesterol change (*mg* per 100 *ml* plasma) ± S.E. for number of rats in parentheses.

†Mean prothrombin rate $\left(\frac{1}{\text{PT (Sec.)}} \times 1000\right)$ ± S.E. for number of rats in parentheses.

tion, on experimental day 36 the prothrombin rate was significantly lowered and both castration ($P<0.005$) and vitamin K_3 ($P<0.01$) increased the prothrombin rate. Although the interaction of castration and vitamin K supplementation increased the prothrombin rates, the values did not reach statistical significance in this experiment. Even though 0.1 *mcg* K_3/g diet significantly increased the prothrombin rate, it was adequate only for the castrated rats. The progressive decrease in prothrombin rate with time due to vitamin K deprivation was significant.

The plasma cholesterol values for the castrated rats at the start of the experiment were higher ($P<0.05$) compared to the intact rats. The rise in plasma cholesterol subsequent to castration is consistent with the findings of other investigators (1, 3, 11). It is interesting that although the plasma cholesterol level increased subsequent to castration, the plasma prothrombin rate was not affected as long as rats were fed rat chow. This indicates that testosterone does not decrease the prothrombin rate when the diet is vitamin K adequate. Analysis of cholesterol changes on experimental day 18 indicated that neither vitamin K_3 nor castration effect was statistically significant. However, vitamin K_3 & castration interaction was significant ($P<0.005$) indicating that vitamin K_3 supplementation at the 0.1 *mcg* level was effective in reducing the plasma cholesterol in castrated rats after 18 days.

The hypocholesteremic effect of vitamin K_3 observed in the castrated but not in the intact rats on experimental day 18 may possibly be due to lower concentration of K_3 required by the castrated rats, or K_3 may be degraded more quickly in the intact rats than in the castrated ones. The other explanation could be that the liver binding capacity for vitamin K_3 may be greater in the castrated than in the intact rats since the vitamin K_3 intake was not different in the two groups.

The hypocholesteremic effect of vitamin K_3 (0.1 *mcg/g* diet) was not significant on experimental day 36. However, the effect of this dose level of vitamin K_3 in maintaining the prothrombin rate in the castrates was significant ($P<0.01$) on day 36. This would indicate that either the vitamin K requirement changed with age or vitamin K deficiency developed due to the depletion of body vitamin K storage in 36 days. The fact that 0.1 *mcg* vitamin K_3 per *g* of diet was adequate to maintain normal prothrombin rates in castrates but had no hypocholesteremic effect after day 36 would indicate that prothrombin synthesis can occur at lower concentration of votamin K than that required for a hypocholesteremic effect. The effect of vitamin K_3 in increasing the prothrombin rates has been reported both in the castrated and intact rats in separate experiments (6). It has also been reported that the castrated rats were less susceptible than the intact rats to hemorrhagic diathesis when fed hemorrhagic irradiated beef diet (6,7). It is, however, not clear whether the decreased susceptibility of the castrated rats to hemorrhages was due to the lack of testosterone as such or due to changes in vitamin K metabolism possibly effecting the half life of vitamin K_3. The data of this experiment indicated that, while 0.1 *mcg* vitamin K_3 per *g* of diet was adequate to maintain normal prothrombin rate in the castrated rats upto 36 days, this dose level was not adequate for intact rats after 18

days. It was further noted that the prothrombin rates of the castrated rats remained normal up to 18 days on a vitamin K-free diet, while on the same diet vitamin K deficiency was observed in the intact rats after 18 days. These findings indicated that the vitamin K requirement of the intact rat is greater than the castrated rat of the same age and on the same diet. It was reported by Mameesh and Johnson (8) that 0.1 *mcg* K_1 per *g* of diet maintained the plasma prothrombin times of the weanling male rats fed a hemorrhagic diet for a period of 4 weeks. However, it was observed in the present experiment that 0.1 *mcg* menadione per *g* of diet was not adequate to maintain normal prothrombin time in adult intact male rats for a period of 18 days. The increased requirement of vitamin K by the adult compared to weanling rats further support the hypothesis that testosterone increases the vitamin K requirement possibly by decreasing its half life.

TABLE II: *Effect of vitamin K_3 on plasma cholesterol and plasma prothrombin rate of castrated and intact rats fed synthetic diet.*

Experimental Day	*mcg vitamin K_3 per g of diet*			
	0	0.1	0.5	0.5
	Castrate			Intact
	Plasma Cholesterol			
0	89.8 ± 5.54 (4)*	89.5 7.28 (4)	94.8 ± 6.62 (4)	
18	+23.2 ± 10.61 (4)**	+14.2 ± 7.72 (4)	+5.0 ± 3.63 (4)	
36	+19.7 ± 4.93 (3)	— 1.5 ± 10.5 (2)	+6.5 ± 5.38 (4)	
	Plasma Prothrombin Time			
0	74.0 ± 1.22 (4)†	72.8 ± 1.03 (4)	±73.0 ± 3.63 (4)	72.0 ± 2.30 (4)
18	65.8 ± 2.29 (4)	74.8 ± 1.44 (4)	±75.0 ± 1.68 (4)	71.0 ± 1.22 (4)
36	61.5 ± 8.50 (2)	71.5 ± 1.50 (2)	±74.0 ± 3.78 (3)	67.2 ± 1.89 (4)

*Mean plasma cholesterol (*mg* per 100 *ml* plasma) for number of rats in parentheses.

**Mean plasma cholesterol change (*mg* per 100 *ml* plasma) ±S.E for number of rats in parentheses.

†Mean plasma prothrombin rate $\left(\frac{1}{\text{PT (Sec.)}} \text{X } 1000\right)$ ±S.E. for number rats in parentheses.

Since 0.1 *mcg* vitamin K_3 per *g* of diet decreased the plasma cholesterol level only in the castrated rats after 18 days but not after 36 days, vitamin K_3 was added at levels of 0.1 and 0.5 *mcg* per *g* of diet in the second experiment. The mean prothrombin rates as well as the initial mean plasma cholesterol level and the mean changes after 18 and 36 days are given in Table II. Vitamin K_3 at the dose level of 0.5 *mcg* per *g* of diet was adequate to maintain normal prothrombin rate both in the castrated and the intact rats up to 36 days, thus indicating higher vitamin K_3 requirements for the intact rats. The hypocholesteremic effect of vitamin K_3 did not reach statistical significance. Since 0.1 mcg vitamin K_3 per g of diet was found to lower

the plasma cholesterol level of castrated rats after 18 days, both in the first and second experiment, a test of homogeneity was run (14) and statistical analysis showed that the replication effect was not significant. On this basis, the data of two replications for experimental day 18 were combined and analysed. In this test the hypocholesteremic effect of 0.1 *mcg* vitamin K_3 per *g* of diet was found significant ($P<0.05$). Vitamin K_3 at a dose level 0.5 *mcg* per *g* of diet was adequate to maintain normal cholesterol level up to 36 days in the castrated rats.

TABLE III: *Effects of vitamin K_3 and testosterone propionate on plasma cholesterol and plasma prothrombin rate of castrated and intact rats fed synthetic diet*.*

mg testosterone per week		*mcg vitamin K_3 per g of diet*			
		0	1	0	1
		Castrate		Intact	
		Plasma Cholesterol*			
		+26.2 ± 8.58**	+24.7 ± 6.76	+32.3 ± 1.14	+24.7 ± 5.54
0	1.		3.	5.	7.
		78.0 ± 5.32***	78.0 ± 5.13	+61.3 ± 5.77	63.5 ± 3.42
		+5.3 ± 3.63**	—6.0 ± 5.08	+28.0 ± 5.99	+15.2 ± 3.40
10†	2.		4.	6.	8.
		80.2 ± 5.88***	80.5 ± 5.02	65.3 ± 6.08	62.8 ± 3.93
		Plasma Prothrombin Rate*			
0	1.	75.5 ± 6.37††	3. 94.8 ± 2.00	5. 36.0 ± 5.58	7. 82.2 ± 1.38
10	2.	36.6 ± 7.00	4. 90.7 ± 2.20	6. 26.2 ± 4.39	8. 85.0 ± 1.00

*Each value mean of 6 rats.

**Mean plasma cholesterol change (*mg* per 100 *ml* plasma) ± S.E. after 18 days.

***Mean plams cholesterol (*mg* per 100 *ml* plasma) ± S.E. at the start of experiment.

†Injected 5 *mg* twice a week.

††Plasma prothrombin rate $\left(\frac{1}{\text{PT (Sec.)}} \times 1000\right)$ ± S.E. after 18 days. Initial prothrombin rates were not determined.

In the third experiment, 1.0 *mcg* vitamin K_3 per *g* of diet was used to see if a higher dose of vitamin K_3 would decrease the plasma cholesterol level in the intact rats. Testosterone propionate was injected to both the castrated and intact rats at the rate of 10 *mg* per week to determine its effect on plasma cholesterol and prothrombin rate. The initial mean plasma cholesterol level and mean changes of 8 experimental groups after 18 days as well as the mean prothrombin rates for experimental day 18 are given in Table III. The initial plasma cholesterol level of the castrated rats was higher ($P< 0.05$) compared to intact rats as was observed in the first experiment. Testosterone reduced the plasma cholesterol ($P<0.005$); however, a markedly

lower response to testosterone was observed in the intact rats compared to the castrated rats. This lower efficiency of exogenous testosterone in the intact rats could be due to their endogenous testosterone secretion. The fact that 1 *mcg* K_3 per *g* diet had a hypocholesteremic effect in the intact rats along with the observation of experiment 1 that 0.1 *mcg* K_3 per *g* of diet had no hypocholestermic effect in the intact rats but did decrease cholesterol in the castrated rats would indicate that the level of vitamin K required to elicit a hypocholestermic response is lower in castrated compared to intact rats. It was observed that both castration and vitamin K_3 increased ($P<0.005$) while testosterone decreased ($P<0.005$) the prothrombin rates. The interactions of castration & vitamin K_3, of castration & testosterone, and vitamin K_3 & testosterone were significant. These interactions indicated that vitamin K_3 and testosterone have opposite effects on prothrombin rate. Interaction of castration & vitamin K_3 in the first experiment where vitamin K_3 was used at the rate of 0.1 *mcg/g* of diet was not significant. Testosterone had no effect on prothrombin rates of intact rats or castrated rats fed vitamin K in the diet. Thus, it appears that the effect of testosterone on prothrombin rate is dependent on the adequacy of vitamin K in the diet and that the vitamin K requirement is increased in the presence of testosterone.

The hypocholesteremic effect of vitamin K_3 observed in these experiments indicates a possible role of vitamin K_3 on cholesterol metabolism. Although the exact mechanism of the hypocholesteremic action of the vitamin K_3 is not clear, the data presented indicates that testosterone modifies the metabolism of vitamin K_3.

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