RESPONSE OF PROTHROMBIN RATE TO EXOGENOUS TESTOSTERONE IN RATS FED DICUMAROL*

By

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Wide variations in response to dicumarol in humans were observed by Wright and Prandoni (19). Allen (1) reported that identical amounts of dicumarol administered to different people produced dissimilar effects on prothrombin time. He noted that the same dose of dicumarol, administered to a given person on more than one occasion, could affect prothrombin time differently. A marked variation in the sensitivity of individual rabbits to dicumarol was observed by Smith (17). When a hemorrhagenic diet containing irradiated beef was fed, male rats were found to be more susceptible to hemorrhages than female rats (11, 12) and prothrombin time was increased by the administration of testosterone (7). Studies of Coppage and Cooner (4) indicated that plasma testosterone concentration varies from person to person. No published report could be found concerning a possible correlation of dicumarol effects on blood coagulation with circulating plasma testosterone levels. The purpose of the present study was to investigate the response of prothrombin time to various dose levels of exogenous testosterone in rats fed dicumarol.

MATERIALS AND METHODS

Forty-eight-24-week-old rats of the Charles River strain were castrated; Lugol's iodine solution was applied to the site of the incision. After a two week period of recovery the rats were bled for prothrombin time determination, and were distributed into six experimental groups by means of a table of random numbers. All rats were fed a commercial diet[1] until they were bled. The rats were individually housed in cages with raised wire screen bottoms in an air-conditioned room. The rats in groups 1, 2, and 3 were fed a basal diet described by Mameesh and Johnson (9), except that USP Salt Mix XVI was used. The rats in groups 4, 5, and 6 were fed the basal diet to which dicumarol had been added at the level of 40 *mg/kg* of diet. The rats in groups 1 and 4 received one subcutaneous injection of 0.1 *ml* of sesame oil per week. The rats in groups 2 and 5 were injected subcutaneously with 0.5 *mg* of testosterone propionate in 0.1 *ml* of sesame oil per week and the rats in groups 3 and 6 were given 1.5 *mg* of testosterone propionate in 0.1 *ml* of sesame oil per week. All rats were bled after the first and second week of feeding the experimental diets.

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1. Rockland Rat/Mouse diet, Tekland Inc., Monmouth, Illinois

The experimental rations and water were offered *ad libitum*. A sufficient quantity of each ration to last for one week was mixed at one time. The rations were stored in a freezer in plastic bags in order to minimize oxidative processes. The rats were weighed individually once a week. Coprophagy was not prevented. All animals were examined daily for any signs of hemorrhage, anemia or other abnormality. Rats which died spontaneously during the course of the experiment or after being bled were autopsied to examine the site and the extent of hemorrhages. At the end of the experiment all animals were killed with ether and examined for internal hemorrhages.

The rats were anesthetized with ether before being bled by cardiac puncture. One *ml* of blood was obtained for prothrombin time determination. The blood sample was transferred into a glass tube containing a sodium oxalate solution, mixed gently, and centrifuged at 830 x *g*. The plasma was separated and refrigerated until prothrombin time was determined by the method of Quick (13). The time which elapsed between bleeding and prothrombin time determination was less than one hour. Activated thromboplastin [2] was used for prothrombin time determination. All prothrombin time determinations were done in duplicate.

A prothrombin time longer than 20 seconds was considered abnormally long and indicative of hemorrhagic condition. All rats dying of spontaneous hemorrhages as well as those which died after being bled and showed a prothrombin time longer than 20 seconds were counted as deaths due to hemorrhagic condition. Rats which died during anesthesia or bleeding and had a prothrombin time of less than 20 seconds were counted as accidental deaths and were excluded when mortality data was calculated. Prothrombin times longer than 300 seconds were taken as 300 seconds in the statistical analysis. Prothrombin times were transformed into prothrombin rates $\left(\frac{1000}{\text{prothrombin time in sec.}}\right)$ for statistical analysis as used by other workers (7). The results were analyzed by the t-test and analysis of variance with unequal subclass numbers (18).

RESULTS AND DISCUSSION

Preliminary experiments carried out in this laboratory indicated that 40 *mg* of dicumarol per *kg* of diet would be a suitable dose level for a two-week study with rats on blood coagulation.

Five of the 48 rats distributed into the six experimental groups had initial prothrombin times longer than 16 seconds, and were discarded. The group mean prothrombin rates for the remaining 43 rats were found to be statistically equal. Table 1 shows the group mean prothrombin rates at weeks 0,1, and 2. At weeks 1 and 2 a wide individual variation in prothrombin rate was observed within the groups of rats fed the ration containing dicumarol. A comparable variation in prothrombin time has been reported by Mellette and Leone (11).

Dicumarol alone caused a decrease ($p<0.005$) in prothrombin rate at weeks 1 and 2. Testosterone alone at either dose level had no effect on prothrombin rate at weeks 1 and 2.

2. Dade Reagent Inc., Miami, Florida.

However, testosterone in the presence of dicumarol significantly ($p<0.05$) decreased the prothrombin rate at experimental week 2 (see Table I). This effect of testosterone in the presence of dicumarol is an indication of synergism of testosterone and dicumarol since testosterone alone had no effect on prothrombin rate. The dicumarol x testosterone interaction did not reach statistical significance possibly because the number of observations was not large enough due to the high mortality rate. This indication of synergism is reinforced by the trend of the mortality results at week 2 shown in Table I.

TABLE I

Plasma prothrombin rate and percent mortality of castrated rats fed dicumarol and administered testosterone propionate

Group	1	2	3	4	5	6
*Dicumarol**	0	0	0	40	40	40
*Testosterone***	0	0.5	1.5	0	0.5	1.5
Experimental Week						
0	75±2·50(6)†	76±3.46(7)	74±2.58(6)	79±2.68(8)	79±2.28(8)	81±3.76(8)
1	74±3.60(6)	80±3.41(7)	83±0.48(6)	47±5.48(8)	48±8.77(7)	42±9.96(8)
3	86±5.27(5)	77±3.59(5)	77±4.76(5)	33±6.64(5)	26±9.21(4)	8±2.73(3)
Percent Mortality††	0	0	0	25	50	62

**mg* per *kg* diet

***mg* per week per rat

†Mean prothrombin rate $\left(\frac{1000}{\text{prothrombin time in sec.}}\right)$ with standard error of mean for number of rats in parenthesis.

††Percent mortality at the end of week 2; rats which died of accidents were excluded.

Malhotra *et al.* (7) showed that the administration of 0.5 *mg* of testosterone per week for ten weeks caused a decrease in the prothrombin rate of rats fed a diet containing irradiated beef. Testosterone at the dose level of 0.5 *mg* per week had no effect on the prothrombin rate in the present study which differs from that of Malhotra *et al.* (7) in regard to the diet, the strain, the age of the rats, and the duration of the experiment. Since adult rats were reported to be more susceptible to hemorrhagic diathesis than weanling rats (6,10, 11), it would appear that the required dose level of testosterone is dependent on body weight. Differences in strain susceptibility to hemorrhagic diathesis have been reported by other workers (8, 11).

As shown in Table I, at the end of week 2 there was no mortality due to hemorrhages in the three groups fed the basal diet. In the presence of dicumarol the mortality rate tended to rise as the dose level of testosterone was increased. The rats which died spontaneously, on autopsy showed hemorrhages predominantly in the chest cavity. None of the rats which were fed the basal diet with or without administration of testosterone and which died during the

experiment had internal hemorrhages. This shows that, based on prothrombin rates, feeding the vitamin K-free basal diet for a period of two weeks did not produce a vitamin K deficiency state in rats housed in conventional wire screan cages where coprophagy was not prevented. Moreover, this could account for the absence of hypoprothrombopenic effect of testosterone in the absence of dicumarol in this two-week experiment. The observation that testosterone caused a further reduction in prothrombin rate of rats fed dicumarol is consistent with the results of Mallette and Leone (11). These authors reported that there was no effect of testosterone on the prothrombin level of rats fed control diets, but that testosterone lowered the level of prothrombin and factor V in rats fed irradiated beef. Rutherford *et al.* (16) reported that estrogens significantly increased the vitamin K-dependent factors II, VII, IX and X. That testosterone exerts its effect on blood coagulation by lowering vitamin K-dependent coagulation factors is suggested by the work of Dufault *et al.* (5) who found that testosterone decreased the platelet adhesive index and factor IX in humans. The effect of exogenous testosterone in the presence of administered anticoagulants appears to be species specific. This is evident by the absence of activity of androgenic hormones on the prothrombin complex of intact dogs fed dicumarol (15) and from the observation of Charles *et al.* (3) that, when chicks were fed anticoagulants, male chicks had shorter prothrombin times compared to female chicks, which is contrary to the hypoprothrombopenic effect of testosterone observed in rats fed dicumarol in this study. That man appears to respond like the rat is supported by the report of Robinson *et al.* (14) that in humans the dose of anticoagulant (warfarin) had to be reduced when the subjects were simultaneously administered methyl testosterone. Dicumarol and vitamin K both affect clotting factors II, VII, IX and X. The synergistic effect of the combination of dicumarol and testosterone could be due to testosterone lowering some or all of the clotting factors reduced by dicumarol. Testosterone could possibly have a hypoprothrombopenic effect by causing a more rapid turnover of clotting factors, thus making more demand on their synthesis which would deplete vitamin K reserves of the body. Testosterone may also have an interaction with vitamin K or dicumarol at the cellular level which could cause greater metabolism of vitamin K. We have observed (unpublished) that the subcutaneous administration of 5 *mg* of testosterone/ animal twice a week to rats fed the same diet containing dicumarol as used in this study resulted in 100% mortality after the rats were bled at the end of one week. This dose of testosterone, which is about six times the amount used in the present study, slightly decreased the food intake of the rats fed dicumarol but not of rats fed dicumarol-free, diet, thus indicating that the greater susceptibility to hemorrhages of rats given the combined treatment of testosterone and dicumarol is not due to increased dicumarol intake. The combination of testosterone and dicumarol might have caused liver damage but this seems unlikely as Bengmark and Olsson (2) reported that, based on fat infiltration, testosterone propionate reduced susceptibility to liver damage of rats.

The results of the present study, when compared with published results of other studies, indicate that the effect of testosterone on blood coagulation differs from species to species. The endogenous testosterone secretion will not affect the prothrombin time of subjects fed a vitamin

K-adequate diet, but would increase their prothrombin time when they are vitamin K-deficient or when anticoagulants are being administered. The wide individual variation in response to the same dose of dicumarol may be partly due to individual variation in the plasma testosterone level of the recipients of anticoagulant therapy. It must be emphasized that the results are prejudiced against the conclusion reached because of the high mortality rate in rats fed the dicumarol and administered testosterone at one week. These rats would have much lower prothrombin rates at the end of the second week and thus would have increased the statistical significance of the results.

SUMMARY

Forty-eight castrated adult rats of the Charles River strain were used in a two-week experiment to test the possibility that the variable effect of the same dose of dicumarol in different persons may be partly due to individual variation in the circulating plasma testosterone level. The combined effect of dicumarol and testosterone at various dose levels was measured in terms of prothrombin rate and mortality rate due to hemorrhagic diathesis. Testosterone propionate was administered subcutaneously at the level of 0, 0.5, and 1.5 *mg* per rat per week. Dicumarol was fed in the ration at the rate of 0 and 40 *mg kg* of diet. The rats were bled at the start of the experiment and again at the end of the first and second week. Testosterone alone at either dose level did not influence prothrombin time. Dicumarol caused a decrease ($p<0.005$) in prothrombin rate at the end of weeks 1 and 2. In the presence of dicumarol, testosterone caused a decrease ($p<0.05$) in prothrombin rate at the end of week 2. Data indicate a synergistic effect of testosterone and dicumarol on prothrombin rate and mortality rate. These results support the hypothesis that the variable effect of the same dose of dicumarol on prothrombin time in different individuals could be in part due to individual variation in circulatory plasma testosterone level.

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