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Relationship of Estrogen and Vitamin K.* (28055)

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During studies on the nutritional adequacy of irradiated beef, Metta *et al.*(1) observed the widespread occurrence of deaths due to hemorrhage in male rats. Vitamin K supplemented to the irradiated beef diets cured this hemorrhagic syndrome. It was observed that the female rat was more resistant to dietary Vit. K deficiency than was the male(2), and this difference was not due to lower food intake or growth rate of the female rat. Mellette and Leone(3,4) and others(5) have shown that the incidence of hemorrhagic syndrome associated with the feeding of irradiated beef was influenced by the age, sex and strain of the rat. Mellette(4) reported that administration of male hormones or castration of female rats results in an increased susceptibility to hypoprothrombinemia and hemorrhage while the converse is true for administration of estrogenic hormones and castration of the male animal.

We report here the effect of single doses of estradiol and of testosterone on prothrombin levels in rats(6) and chicks maintained on a synthetic Vit. K-free diet.

Another important aspect of this problem is the species difference in susceptibility to dietary Vit. K deficiency. The response of the chick as reported here is entirely different from that of the rat.

Materials and methods. Weanling rats of the Sprague-Dawley strain were maintained in tubular cages to prevent coprophagy throughout each experiment and were fed *ad libitum* a synthetic Vit. K-free diet(2,7).

Day-old chicks were fed a Vit. K-free soya protein-containing diet(2) *ad libitum* for 23 days. Plasma prothrombin times were determined essentially according to Quick(8). Commercial thromboplastin (Difco) was used in the rat experiments and acetone dehydrated chick brain powder was used as thromboplastin source in the chick experiments.

Results. 1. Estrogen administration, (a) Rat results. In a preliminary trial we observed that when estradiol was administered intramuscularly (at a dose of 1 mg/rat) to male rats after 24 days on the Vit. K-free diet, prothrombin times were restored to normal in 4 out of 6 rats, while the negative controls had the usual high prothrombin times

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TABLE I. Effect of Estradiol on Plasma Prothrombin Time of Male Rats Maintained on Vit K-Deficient Diet.

Group	No. of rats	Treatment	Plasma prothrombin time (sec)
			Mean ± standard deviation of mean
A	4	Injected .5 ml corn oil/rat	108 ± 7.0
B	4	Injected 20 μg vit K_1/rat	14 ± 2.7
C	5	Injected 100 μg estradiol in 5 ml corn oil	16 ± 1.4
D	5	Injected 1 mg estradiol in .5 ml corn oil	17 ± 1.7

Weanling male rats were maintained on a synthetic vit K-deficient diet for 3 weeks. The rats were sacrificed 24 hr after injection (I.M.) for plasma prothrombin time determination.

(see Table IV, Ref. 4). Administration of menadione was effective in restoring prothrombin times to normal values. This led us to a series of experiments to determine the minimum effective dose of estradiol against Vit. K deficiency and time required from injection to response.

Data in Table I show that estradiol administration at a dosage of 100 μg or 1 mg/rat (Groups C and D) was equally effective in bringing about normal prothrombin time (about 14-16 secs mean) comparable to those of the control group given 20 μg Vit. K_1 (Group B). This effect was apparent 24 hours after the hormone administration. Further data (Table II) obtained in a similar study show that at a dosage of 10 μg/rat there was a decrease in prothrombin time (to about 53 secs mean), while at 20 μg or 50 μg/rat (Groups D and E) the prothrombin times (25, 26 secs) were comparable to the controls given K_1 (Group B). The negative controls had very high prothrombin times (greater than 120 secs Group A). This effect was observed 6 hours after administration of the estrogen.

(b) Chick results. These findings are in contrast with those observed in studies using the chick as the experimental animal. We have routinely used female chicks for bioassay of Vit. K. Since, however, the female rat shows a marked resistance to Vit. K deficiency the comparative susceptibility of the male and female day-old chick was studied. Fifty male and 50 female one-day-old chicks were fed the Vit. K-free chick diet(2) for 15 days. At 5, 10 and 15 days, respectively, 15 chicks of each sex were sacrificed and their plasma prothrombin times determined. While prothrombin times increased progressively each period, no differences were obtained in prothrombin times between the male and female chicks at any of the time intervals.

Subcutaneous administration of estradiol even at a dosage of 1 mg/chick was essentially ineffective in restoring prothrombin times to normal (Table III). However, it may be that in the 3-week-old chick hormonal

TABLE II. Effect of Estradiol Administration on Plasma Prothrombin Time of Male Rats on Vit K-Deficient Diet.

Group	No. of rats	Treatment	Plasma prothrombin time (sec)
			Mean ± standard deviation of mean
A	6	None	120 +
B	5	Injected I.M. vit K_1 in .5 ml corn oil	30 ± 4.5
C	7	Injected I.M. 10 μg estradiol in .1 ml corn oil	53 ± 11.0
D	5	Injected I.M. 20 μg estradiol	25 ± 2.7
E	6	Injected I.M. 50 μg estradiol	26 ± 1.4

Weanling male rats were maintained on vit K-deficient diet for 3 weeks. Rats were sacrificed 6 hr after injection for plasma prothrombin time determination.

TABLE III. Effect of Estradiol on Plasma Prothrombin Time of Chicks Maintained on Vit K-Deficient Diet.

Group	No. of chicks	Sex	Treatment	Plasma prothrombin time (sec) Mean (Range)
A	4	♂	—	>180
B	4	♀	—	>180
C	4	♂	Injected 100 μg K_1 in .5 ml corn oil	28 (25- 29)
D	4	♀	*idem*	27 (24- 30)
E	5	♂	Injected 1 mg estradiol in .5 ml corn oil	96 (70-119)
F	6	♀	*idem*	>180

Day-old chicks were maintained on a synthetic vit K-deficient diet for 23 days and were sacrificed 26 hr after subcutaneous administration of vit K_1 or estradiol for plasma prothrombin time determination.

TABLE IV. Effect of Testosterone on Plasma Prothrombin Time.

Group	No. of rats	Exp. I Treatment	Plasma prothrombin time (sec)
			Mean ± standard deviation of mean
A	6	Injected I.M. .5 ml corn oil/rat	14 ± .5
B	12	Injected I.M. 1 mg testosterone propionate/rat in .5 ml corn oil	21 ± 2.0

Female rats were maintained on the synthetic vit K-free ration for 24 days. 15 hr after the injection they were sacrificed and plasma prothrombin time determined; ($P > .01$).

balance is not fully operative, and further study is warranted in this area.

2. Androgen administration. In contrast to the effect of estrogen administration to male rats, administration of the male hormone to female rats was observed to bring about significant hypoprothrombinemia. The data in Table IV show that testosterone propionate at a dose of 1 mg/rat increased the prothrombin time of female rats significantly (an average of 21 secs) as compared to untreated controls (14 secs). This difference was significant at the 1% level.

Discussion. Mellette and Leone(3,4) showed the effects of repeated administration of estrogen in providing some measure of protection against the hemorrhagic syndrome. In their investigations of adult male rats fed an irradiated beef diet (*i.e.*, Vit. K-deficient) administration of 50 μg of estrogen at 3-day intervals protected 3 out of 5 rats compared to 100% mortality in the control group. They noticed no substantial changes in either factor V or fibrinogen. They pointed out that the effects of estrogen administered to animals on *ad libitum* diets are difficult to evaluate because of anorexogenic effects and a resultant decrease in food consumption and weight gain. However, by pair-feeding experiment(2) we showed that sex effect on Vit. K deficiency in the rat was not due to differences in food intake.

Mellette and Leone(3) found no substantial effect of estrogen on factor V or fibrinogen levels which would imply that the rapid response to estrogen which we have observed is due to a "direct" effect on prothrombin activation.

Our present studies, on the other hand, show a rapid response to a single injected dose of the hormone. In this case, the anorexogenic effect of the hormone is not involved.

In studies on increasing the susceptibility of female rats to hemorrhage by injection of androgens, Mellette and Leone(3) reported that animals (on an irradiated beef diet) receiving 1 mg of testosterone propionate twice weekly showed a gradual decrease in prothrombin levels. Again, as in the case of estrogen, we have observed a significant response, but in this case an increase in prothrombin time after a single dose of testosterone injection indicates again some kind of "direct" effect on prothrombin activation.

The lack of sex difference in the chick in regard to susceptibility to Vit. K deficiency might logically be considered as being due to lack of sexual development at this age. However, the lack of response to the injection of estrogen certainly argues against a "direct" role of estrogen in replacing Vit. K for prothrombin activity and suggests that the activity of the hormones in the rat must be through the existing hormonal pattern of the more mature animal.

On the other hand, Schjeide *et al.*(9) have reported a stimulatory effect of estrogen on plasma protein synthesis by the liver of the chick at the embryo stage.

Summary. Estradiol injection into male rats kept on a Vit. K-free diet was effective in lowering plasma prothrombin time to normal values in 6 hours. This Vit. K-like activity was observed even at a dosage of 20 μg/rat. Conversely, testosterone administration (1 mg/rat) to female rats significantly increased plasma prothrombin time as compared to untreated controls.

In contrast to the results observed with rats, male and female chicks were equally susceptible to dietary Vit. K deficiency, and estradiol administration, even at a dose of 1 mg/chick, did not alleviate the hypoprothrombinemia.

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Comparative Study of Effect of Epinephrine and Norepinephrine on Cardiovascular System of Turtle, Alligator, Chicken and Opossum.* (28056)

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Our knowledge of the phylogenetic development of the control of the cardiovascular system is fragmentary. Relatively little is known about the role of humoral agents in the control of blood pressure in the lower vertebrates. Lutz(1) and Mott(2) have shown that small doses of epinephrine cause prolonged rises of blood pressure in elasmobranchs and eels. Recently, attention has been focused on the circulation and hemodynamics of the reptilian heart. There have been many studies of the reptilian heart, but relatively few correlating structure and function. White(3) studied the blood oxygen levels in the aorta, atrium and pulmonary artery and showed relatively complete separation of aerated and non-aerated blood in the heart of *Caiman sclerops.* Steggerda and Essex(4) recorded simultaneously the cardiac and arterial pressures of the turtle. The circulatory dynamics of the 3-chambered snake heart have been studied(5,6). To our knowledge there have been no studies on the effects of catecholamines in the reptilian heart, and few in the chicken(7). According to the data available, the effects appear to be the same as in the cat and dog, for which there is much experimental evidence. This report, therefore, concerns the comparative reactivity of turtles, alligators, opossums and chickens to epinephrine and norepinephrine.

Materials and methods. All animals were anesthetized with chloral derivatives. In the reptiles, chloral hydrate (1 mg per g of body weight) was administered intraperitoneally. In the chicken and opossum the animals were anesthetized with chloralose (100 mg/kg) dissolved in polyethylene glycol 200 (Carbowax), injected intravenously. After induction of anesthesia in the turtles (*Graptymys geographica*), the cephalad third of the plastron was removed and the brachial artery was cannulated with a PE 100 polyethylene catheter for blood pressure recording. Alligators (*Alligator mississipiensis*) were prepared by a longitudinal incision made in the ventral aspect of the neck and cannulation of the carotid artery with a PE 100 polyethylene catheter for blood pressure recording. In the opossum (*Didelphis virginianis*), a hind leg vein was exposed under ether for injection of chloralose. The carotid artery was exposed in the neck and cannulated with a PE 190 polyethylene catheter for blood pressure recording. Epinephrine and norepinephrine were administered intravenously in all species. Records of the pressure pulse, blood pressure and heart rate were obtained by means of a Statham P23A transducer coupled to a Grass Polygraph. The body tempera-

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