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Role of Vitamin A in Induction of Vitamin K Deficiency in the Rat.* (27128)

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It has been recognized since 1944(1) that hypervitaminosis A in the rat leads to hemorrhages and hypothrombinemia which can be prevented by administration of Vit. K(1,2). The amounts of Vit. A used were many times the amount required for maintenance of the experimental animals.

Our interest in the possible effects of smaller amounts of Vit. A in development of symptoms of Vit. K deficiency in the rat arose from data obtained with 2 diets similar in all respects except for the levels of several micronutrients. The basic diet contained cooked ground beef and had been used by Metta *et al.*(3) in their original observation of its hemorrhagenic effect in male rats. Some modification in our hands gave a diet which produced little evidence of a coagulation defect. The difference in supplemental levels of Vit. A seemed most significant.

In view of the speculation concerning the biological role for Vit. A acid(4), the effect of this compound in induction of hemorrhage was also studied.

Methods. Rats of the St. Louis University colony were individually caged in suspended one-half inch mesh stainless wire cages and given sufficient fresh diet at either 2- or 3-day intervals to allow *ad libitum* feeding. Ten animals were used in each experimental group. Samples of blood were taken by cardiac puncture under light ether anesthesia and the plasma examined by the viper venom procedure of Hjort *et al.*(5). Although the method may detect changes in concentration of both prothrombin and Stuart factor(6,7), data are given here as the per cent of normal prothrombin concentration. Frozen aliquots of pooled plasma from male rats maintained on Purina laboratory chow were used as normal controls and were assayed with each group of experimental samples. Any value less than 85% of the value for normal controls was taken as indicative of experimentally induced hypoprothrombinemia.† The average and range of observations and number of normal animals (prothrombin concentration greater than 85% of normal) found among the total examined are given as criteria of the effect of the diet. Evidence of spontaneous hemorrhagic death is recorded, but other causes of death or loss such as cardiac puncture are not indicated.

In earlier experiments a feeding duration of 8 weeks was used beginning with weanling rats. Subsequently we devised a more convenient assay for potentially hemorrhagenic diets using adult animals 13 weeks of age. As Mellette and Leone(8) have reported, these animals were more susceptible so that a feeding duration of only 2 weeks reflected the nutritional adequacy of the diet. Samples of cardiac blood were taken for analysis after one and 2 weeks on the experimental ration.

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† This value is based on examination of the plasma of 45 adult male rats maintained in the colony on Purina laboratory chow; mean concentration of prothrombin was 99.4 ± 7.5 (s. d.) % of the pooled plasma control.

TABLE I. Effect of Vitamin A Acetate on Prothrombin Concentration in Weanling Male Rats.

Vit. A* (i.u./g)	Purified diet† Prothrombin§ (% of normal)	Normal animals	Irradiated beef diet‡ Prothrombin§ (% of normal)	Normal animals	Fresh beef diet‡ Prothrombin§ (% of normal)	Normal animals
0	85 (69-107)	4/10	101 (79-121)	8/10	101 (85-112)	10/10
.5	96 (69-116)	8/10	75 (44-100)	5/10	92 (70-103)	9/10
5	88 (44-119)	7/10	39 (7- 71)	0/9	92 (75-119)	7/10
50	66 (6-119)	4/10	36 (6-105)	1/10	82 (53-104)	4/10
500	15 (8- 36)	0/10	10 (1- 42)	0/8 ‖	17 (7- 41)	0/10

* Crystalline vit. A acetate (Nutritional Biochemicals, Inc.); beef diets were supplemented as indicated on a dry wt basis.

† The purified diet contained: Labco casein, 21; salts 446, 4; corn starch, 43; glucose, 22; vitaminized glucose, 5; corn oil (Mazola), 5; vit. E acetate, 0.088; viosterol, 0.027. Mineral mix 446 is that of Mameesh and Johnson(9). The vitaminized glucose supplied the following vitamins in mg/100 g of diet: thiamine, 1; riboflavin, 1; calcium pantothenate, 5; pyridoxine, 0.5; niacin, 2; folic acid, 0.1; B_{12}, 0.05; biotin, 0.01; p-aminobenzoic acid, 1; inositol, 25; choline chloride, 250.

‡ The beef diets were essentially the same as those reported by Metta *et al.*(3), except that wheat flour was replaced by corn starch and cod liver oil was replaced by crystalline vit. A acetate as indicated in the table and viosterol (270 i.u. % on a dry wt basis) in a constant amount of corn oil (1.5%).

§ Samples of plasma were examined by the viper venom procedure after an 8-wk feeding duration to weanling male rats. Ranges of values are given in parentheses. Normal animals had more than 85% of control prothrombin concentration (see text).

‖ Two animals died at 7 wk; both showed internal hemorrhage at autopsy.

In general, deviation of individual animals from the mean of a group was similar at both one and 2 weeks. Thus, the reported ranges of observations within experimental groups are probably due to animal variation. In part, this variation may be the result of coprophagy by some of the animals.

Results and discussion. The hemorrhagenic effect of various amounts of crystalline Vit. A acetate in 3 different diets during an 8-week experiment on weanling rats is shown in Table I. Several groups of animals maintained on Vit. K deficient diets slightly modified from those reported here gave similar results. The animals which received the purified diet unsupplemented with Vit. A were moribund in terminal stages of Vit. A deficiency at 8 weeks. All other groups of animals including those receiving excessive amounts of Vit. A gained weight satisfactorily. Vit. A was most effective in diets containing irradiated beef‡; as little as 0.5 i.u./g (dry weight) had a marked effect. Response of the animals to fresh beef and purified diets was similar; more Vit. A was required for marked hypoprothrombinemia. Addition of 2 μg of Vit. K_1 diphosphate§ per gram of purified diet completely protected against the hemorrhagenic effect of 500 i.u. of Vit. A.

The observed greater resistance of female rats to development of symptoms of Vit. K deficiency(10) led us to determine the effect of Vit. A in weanling female rats. No marked difference between the effect of irradiated beef and casein diets containing 500 i.u. of Vit. A acetate per gram was observed in female rats. Furthermore, the effect of Vit. A was much less severe in female than in male rats; however, quantitative evaluation was difficult because of the supernormal response to the venom assay of plasma from adult female rats on control diets.‖

The use of adult male rats for a 2-week experimental period permitted examination of diets deficient in both Vit. A and K without causing the emaciating symptoms of Vit. A deficiency which occurred in weanling animals after 8 weeks. The effects of a purified

‡ Beef used in these studies was purchased by Office of the Quartermaster General. Beef was irradiated at 5.58 megarads by ^{60}Co at the Arco or Savannah River Plants.

§ Generously supplied by Merck Sharp and Dohme.

‖ Ten adult female rats were maintained on the purified casein diet containing no added vit. K and 50 i. u. of vit. A acetate per gram. After one and 2 weeks mean prothrombin concentrations as indicated by the venom assay were 131 and 122% of normal male plasma.

TABLE II. Response of Adult Male Rats to Vitamin A and Vitamin A Acid.

Vit. A (i.u./g)*	Vit. A acetate		Vit. A acid	
	Prothrombin† (% of normal)	Normal animals	Prothrombin† (% of normal)	Normal animals
0	100 (47-131)	16/20		
5	76 (44-120)	8/20	61 (39- 85)	1/10
50	75 (31-111)	8/20	11 (5- 23)	0/10
250	51 (24-117)	2/10	10 (5- 18)	0/10
250‡	108 (60-121)	9/10	84 (49-112)	5/10

* The acetate of vit. A or its carboxylic acid derivative was added to the purified diet described in Table I except that cerelose replaced glucose. The acetate was added to the diet as a commercial concentrate (10^6 i.u./g, Nutritional Biochemicals, Inc.); vit. A acid (Distillation Products Industries) was added to 3 kilos of diet as the sodium salt in no more than 10 ml of 20% aqueous ethanol. For the purpose of recording the data in international units, the acid and acetate were presumed equivalent on a molar basis(11).

† These data were obtained after 2 wk on the experimental ration. Ranges of values are given in parentheses. Normal animals had more than 85% of control prothrombin concentration (see text).

‡ 2.5 μg vit. K_1 diphosphate/g of diet.

Labco casein diet deficient in both vitamins on adult animals are shown in Table II. Almost no evidence of a coagulation defect was observed. The hemorrhagenic effect of Vit. A acetate and Vit. A acid in adult male animals is also shown in Table II. Five units of either compound per gram of diet produced symptoms of Vit. K deficiency. More Vit. A did not markedly increase the hemorrhagenicity of the diet whereas 50 units of the acid produced severe hypoprothrombinemia. Addition of 2.5 μg of Vit. K_1 diphosphate per gram of diet protected against the effect of 250 i.u. of Vit. A and gave partial protection against 250 i.u. of its carboxylic acid derivative.

These studies indicate that the requirement for Vit. K in the rat(12,13) may change with the level of dietary Vit. A. Although Vit. A was more effective in irradiated beef diets (Table I), the influence of 5 i.u. per gram was clearly evident in adult animals on a Labco casein ration¶ (Table II).

The greater hemorrhagenicity of Vit. A acid may be indicative of a biological function for this compound as suggested by several investigators(4) on the basis of other evidence. Earlier studies led Quick(14) to propose that the effect of Vit. A in induction of hemorrhage may be intestinal rather than systemic. In view of the possible biochemical relationships between Vit. A or Vit. A acid and Vit. K, further studies are desirable to determine more clearly the nature of this effect.

Summary. Evidence is presented to indicate that the level of dietary Vit. A is closely related to development of symptoms of Vit. K deficiency in the rat. Under appropriate experimental conditions, 0.5 and 5 i.u. of Vit. A per gram of diet gave reduced prothrombin concentrations which were not observed on corresponding rations deficient in Vit. A. This extends the previously known hemorrhagenic toxicity of Vit. A to physiological levels and emphasizes the need for careful consideration of the level of Vit. A in studies of Vit. K deficiency in the rat. The effect of Vit. A was more severe in male than in female rats. In further studies with male rats, Vit. A acid was markedly more hemorrhagenic than Vit. A acetate.

¶ We have examined the effect of other dietary proteins on development of vit. K deficiency. Results obtained after extraction of the proteins or after supplementation with vit. K. or amino acids indicate a fundamental role for protein nutrition in the maintenance of coagulation factors in the rat. These studies will be published later.

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Changes in Renin Content in Kidneys of Renal Hypertensive Rats. (27129)

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In earlier studies on rats with renal hypertension due to unilateral clamping of the renal artery, the renin content of the clamped kidney was found to be increased, while renin had nearly disappeared from the untouched kidney(1). This unequal distribution of renin was also observed in rats, in which the unilateral clamping had not led to hypertension(1), and also in other species like the rabbit, the cat or the dog, in which it is generally necessary to place clips on both renal arteries in order to obtain hypertension. If both renal arteries are clamped in the rat, the renin content is always lower in the heavier kidney than in the lighter one, even if the difference in weight between the 2 kidneys is small(2). In the above studies the renin content of the kidney was estimated semiquantitatively by injecting a freshly prepared kidney extract into a nephrectomized assay rat. A more precise estimate of renin concentration in kidney tissue, as used here, may be obtained by the indirect assay method, *i.e.*, by measuring the amount of angiotensin liberated from a given excess of angiotensinogen (rat plasma) by the renin contained in a fixed volume of kidney extract. By this procedure it is possible to follow quantitatively the changes in renin concentration in the clamped and unclamped kidney when either the one or the other kidney is removed. The present report concerns a) the influence of unilateral clamping of a renal artery on concentration of renin in the clamped and unclamped kidneys, b) the influence of one kidney on concentration of renin in the other, c) time necessary for the increased or decreased renin content of a kidney to return to normal after removal of its partner.

Methods. Male white rats averaging 160-180 g in weight were made hypertensive by placing a silver clip (0.2 mm I.D.) on either one or both renal arteries. The following groups were investigated 3 to 4 weeks after clamping or after unilateral nephrectomy: a) Unilateral (left) clamping of renal artery, the other kidney being untouched (50 animals); b) clamping both renal arteries (8 animals); c) clamping the left renal artery immediately after removal of the right kidney (16 animals); d) unilateral nephrectomy (25 animals); e) control animals (80 animals).

In a second series of experiments, the time necessary for the renin content to return to normal was investigated. Renin concentration in the kidney was determined before unilateral nephrectomy and at intervals of 2, 8, and 24 days after operation. The following groups were studied: f) Unilaterally nephrectomized normotensive animals; g) renal hypertensive animals after removal of the clamped kidney; h) renal hypertensive animals after removal of the unclamped kidney.

Each group consisted of 15 animals. Subgroups of 5 animals each were sacrificed at the given time intervals.

Renin was determined indirectly accord-