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Influence of Vitamin E on Platelet Aggregation and Thrombocythemia in the Rat (38787)

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It has been proposed that the endoperoxide intermediate in the synthesis of PGE_2 from arachidonate triggers platelet aggregation (1). This peroxide intermediate has been referred to as LASS (1), PGR_2 (2) or PGH_2 (3). Arachidonic acid peroxidized with lipoxidase induces platelet aggregation (4) and may "catalyze" the synthesis of arachidonate to LASS in platelets (5, 6). Antioxidants, including tocopherol, will inhibit prostaglandin synthesis *in vitro* (6). Vitamin E (α-tocopherol) prevents the formation of peroxides in adipose tissue *in vivo* (7) and may function as an *in vivo* antioxidant in other tissues as well (8). If peroxidation of arachidonate in platelets is inhibited by vitamin E, platelet aggregation may also be inhibited. The following experiments support this hypothesis.

Experimental. Weanling male Sprague–Dawley rats were fed a vitamin E deficient diet high in linoleic acid (9). The plasma tocopherol levels (10) of these rats were less than 100 μg/100 ml and peroxidative red blood cell hemolysis values (11) were over 97%. Therefore, these animals would be considered E-deficient. A control group received 200 mg of *dl*-α-tocopherol acetate per kg of diet. Their plasma tocopherol values were over 1000 μg/100 ml and the peroxidative red blood cell hemolysis value less than 5%. Ten ml of blood was taken from the abdominal aorta with a silanized syringe containing 0.4 ml of heparin (600 units/ml) while animals were under Penthrane anaesthesia. A small aliquot was removed for determination of hematocrit. The blood was transferred to a siliconized glass tube and centrifuged at 950 rpm in a clinical centrifuge. The platelet count was determined with a Coulter counter (12). Platelet aggregation was measured with a chronolog aggregometer using 0.5 ml of the platelet-rich plasma. The instrument was adjusted to 100% transmission with platelet poor plasma and for 0% with platelet rich plasma. Collagen and ADP were used as aggregating agents. The results of two experiments are given in Table I. Unlike human platelet aggregation, in the rat, the first and second waves are not distinguishable with ADP as the aggregating agent. Typical aggregation curves are presented in Fig. 1.

Results and Discussion. Platelet aggregation was less in blood of animals receiving vitamin E than in blood from deficient animals. The difference was highly significant with collagen as the aggregating agent but was not with ADP. With older rats (15–16 wk), the platelet count was significantly higher in deficient animals than in those fed vitamin E. This increase in count could contribute to the increased aggregation. Melhorn and Gross (13) have observed an elevated platelet count in infants with low serum tocopherol levels (less than 400 μg/100

TABLE I. INFLUENCE OF VITAMIN E ON PLATELET COUNT AND PLATELET AGGREGATION.

Age (weeks)	Diet	% Platelet Aggregation: ADP (0.5 μM)	% Platelet Aggregation: Collagen[c] (0.9 mg/ml)	Platelet Count: Cells per $mm^3 \times 10^{-3}$
9–10	Deficient (11)[a]	68.1 ±9.2	74.5 ±6.2**	370 ±35
	Control[b] (11)	46.7 ±6.1	47.8 ±6.1	332 ±33
15–16	Deficient (13)	56.6 ±6.4	74.5 ±7.0**	520 ±24**
	Control (13)	44.1 ±6.4	27.8 ±9.3	268 ±8

[a] Number of animals per treatment.

[b] 200 mg dl-α-tocopherol acetate per kg of diet.

[c] Bovine Achilles Tendon, Type I, Sigma Chemical Co., St. Louis, Mo.

** Significantly different from control, $P < 0.01$.

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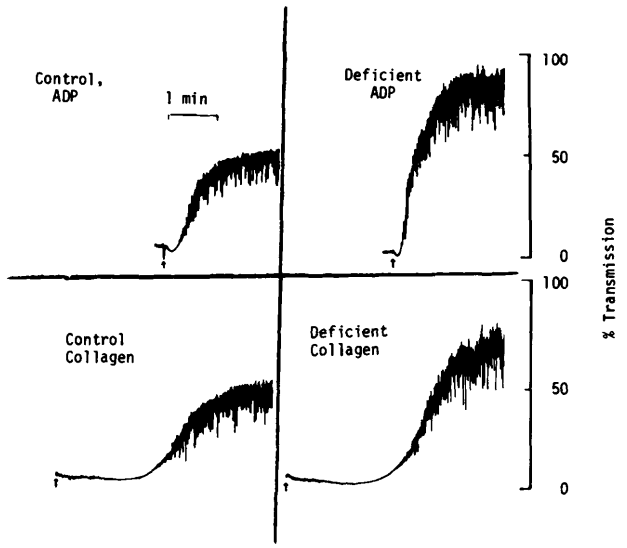

FIG. 1. Typical aggregation curves of platelet rich plasma from control (200 mg of vitamin E per kg of diet) and vitamin E-deficient rats. ADP (0.5 μM/ml) or collagen (0.9 mg/ml) were added at time indicated by the arrows.

ml). Platelet counts were reduced to normal levels following administration of vitamin E. Platelet counts were also reduced following vitamin E administration to adults (14).

In the present study, the platelet count of younger (9–10 wk) deficient animals was the same as control animals. Therefore, the increased aggregation in this age group could not be attributed to a difference in count. The reduction of aggregation by vitamin E could be related to an antioxidant role in prevention of peroxidation of arachidonate. However, chemical evidence for this hypothesis is required. It will be of considerable interest to determine whether the effects of vitamin E on platelet aggregation and platelet count are involved in the etiology of vascular pathology found in vitamin E deficient states (12–17) or in the development of thrombosis in humans.

Summary. Collagen-induced platelet aggregation was increased in 9–10 wk old vitamin E deficient rats although there was no difference in platelet count between deficient and control animals. With a more prolonged deficiency (at 15 wk) both platelet aggregation and platelet counts were elevated in the vitamin E deficient animals.

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