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Prevention of Traumatic Bleeding by Ellagic Acid in Rats.* (30479)

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Ellagic acid activates Hageman factor *in vitro* and *in vivo*(1,2) and causes a state of hypercoagulability in several animal species(2,3). This state of hypercoagulability is characterized by slight shortening of the glass clotting time and marked shortening of the silicone clotting time(2,3), increased prothrombin consumption(2), shortened partial thromboplastin time(2), and acceleration of thromboplastin generation(2,3). It was also noted that normal dogs, rabbits and cats when treated with ellagic acid showed decreased oozing from raw surfaces and from the site of venipuncture when compared with controls(3).

The purpose of this study was to evaluate the effect of ellagic acid on bleeding in the rat as determined by surgical removal of the terminal part of the tail.

Materials and methods. Female white rats (103), Carworth Farms, 350 to 500 g, were maintained under the same conditions of diet (Purina Chow) and environment.

Ellagic acid (4.4′, 5.5′, 6.6′ hexahydroxydiphenic acid 2.6, 2′6′ dilactone) (K & K Lab., Jamaica, N.Y.): dissolved in 0.025M sodium barbital buffer in 0.125M sodium chloride (pH 7.5). A solution with a concentration of 2×10^{-4}M was used.

Procedure. Anesthesia: Intraperitoneal sodium pentobarbital. 15 mg/animal.

The femoral vein was exposed surgically for injection of solutions. Fifty-three animals were injected with 1 ml of the ellagic acid solution per 100 g of body weight in 5 minutes. Fifty animals (controls) were injected with an equal amount of buffer in 5 minutes.

Immediately after the administration of ellagic acid or buffer the tail of the animal was amputated 5 cm from the tip. The animals were then placed on a flat surface with the tails hanging down into a test tube containing 5 mg of EDTA.

The amount of blood loss was measured 30 minutes after the tail wound had stopped bleeding. All animals were then kept under observation for 2 weeks for possible recurrence of bleeding from the site of amputation.

Results. The average blood loss observed in control animals was 4.4 ml while the average blood loss in ellagic acid treated was 0.3 ml. The distribution of the single values of blood loss and the means obtained in the 2 groups of animals are shown in Fig. 1. The difference between the 2 means was highly significant: ($t = 18.6$, $P<0.001$).

There was minimal overlap in the amount of blood loss. All control animals lost more than 1 ml of blood. Only 4 of the ellagic acid treated animals lost more than 0.9 ml of blood. In every case the blood loss with ellagic acid was well below the mean blood loss of the control animals (Fig. 1). In 14 of the 53 ellagic acid treated animals there was no blood loss, *i.e.*, not one drop of blood from the cut tail.

The duration of bleeding was 0 to 10 minutes in ellagic acid treated animals and 10 to 45 minutes in the controls.

No recurrence of bleeding was observed in any ellagic acid treated animal once the

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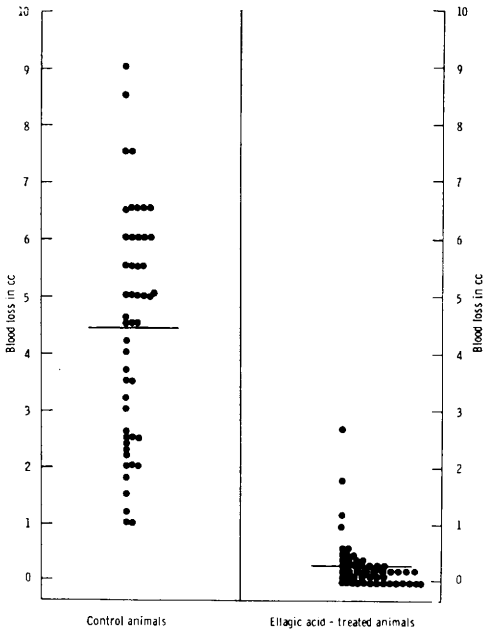

FIG. 1. Distribution of blood loss values (in ml) in control and ellagic-treated animals. Horizontal bars represent the mean in each group.

hemorrhage had stopped, during the 2-week period of observation. No untoward side effects of ellagic acid were noted.

Discussion. Ellagic acid induces increased coagulability presumably by activating Hageman factor(1,2,3). In the course of our experiments(3) it was noted that bleeding from vessel punctures was less in the ellagic acid treated animals than in controls.

The present controlled study has confirmed that ellagic acid in small amounts prevents bleeding, at least from the rat's tail, even though the duration of activity of ellagic acid is short in the rat (about 20 minutes) (3).

Presumably ellagic acid prevents bleeding by activating the coagulation mechanism at an early stage. Other mechanisms are possible but seem unlikely. Vasoconstriction could prevent bleeding but activation of Hageman factor should elicit vasodilation(4), and furthermore, with vasoconstriction only, there is usually delayed bleeding which was not seen in these animals. Ellagic acid might induce platelet plug formation at the site of vessel injury, a common method of control of hemorrhage(5,6) but there has been no evident effect of ellagic acid on platelet function(2,3).

The lack of toxicity and the failure to produce marked changes in the clotting mechanism, which might produce other complications, makes this a most interesting compound.

Summary. Intravenous injection of ellagic acid causes marked statistically significant decrease in bleeding after amputation of the tail in the rat.

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Growth Hormone-Releasing Activity of Crude Ovine Hypothalamic Extracts. (30480)

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Although it is now apparent that the hypothalamus exercises a controlling influence over the secretion of anterior pituitary hormones, evidence for hypothalamic control of growth hormone (GH) secretion has been slow to accrue. This is probably related to the complexity of the growth process. The finding of stunted growth after hypothalamic lesions by itself is not sufficient proof that secretion of GH is impaired(1,2). Reichlin(1) has re-

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