Plasminogen Activator in Mouse Uterine Fluid: Its Suppression by Estradiol and Progesterone.* (31357)

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The widespread use of estrogens and progestationals and their possible relationship to thromboembolic phenomena(1) lends special significance to biologic observations establishing a relationship between hormones and coagulation or fibrinolytic systems. Our studies show that female sex hormones suppress an activator of plasminogen in mouse endometrial fluid and thereby inhibit its fibrinolytic activity.

Increased fibrinolytic activity of blood and tissues is due to increased levels of plasminogen activator(2). A great variety of stimuli cause an increase in blood plasminogen activator activity(2). It is less clear what factors increase or suppress tissue activator activity. Human and animal endometrial tissue extracts are particularly rich in a plasminogen activator(3,4). Several investigations suggest a relationship between circulating concentrations of female sex hormones and endometrial plasminogen activator activity. Fibrinolytic activity of the endometrium varies during the menstrual cycle, and is absent before menarche and after menopause(3-6). In castrated rats treated with estrogens, an increase in endometrial fibrinolytic activity correlates with falling blood levels of estrogens(5).

An activator of human and bovine plasminogen has been described in the sterile mouse uterine fluid accumulating following cervical ligation(7). This activator is stable at —20°C, and is destroyed by heating to 70°C for 30 minutes at neutral pH. The following experiments demonstrate that estradiol and progesterone given to mice during the accumulation of uterine fluid affect its chemical composition and suppress uterine plasminogen activator activity.

Methods. Bio-Swiss mice underwent cervical ligation as previously described(8). Three groups of 10-12 mice each received either subcutaneous injection of 1 μg estradiol benzoate in 0.1 ml sesame oil daily, or implantation of a pellet containing progesterone acetate from which approximately 0.5 to 1.0 mg was absorbed daily. A control group received sesame oil alone. The contents of the distended uterine horns were aspirated before hormone administration and on the 21st, 42nd, and 63rd days of the experiment and the fluid volumes were measured.

The aspirates were cultured, passed through a Millipore® filter and stored at —20°C until assay. Infected fluids were discarded. The protein and carbohydrate concentrations were determined by previously described methods(9,10). Plasminogen activator activity was measured in duplicate, utilizing plasminogen-rich bovine fibrin plates(11). The area of lysis caused by 0.03 ml of the sample at 37°C for 18 hours was expressed as the product of two perpendicular diameters in mm^2.

The plasminogen activator was concentrated and partially purified from pooled mouse uterine fluid at 45% ammonium sulfate concentration at pH 8.0 and 0°C. This semi-purified activator was used to test for possible inhibition of plasminogen activation by the endometrial fluid obtained during hormonal treatment. For this purpose, serial dilutions of the activator were added to a constant concentration of fluid from estrogen-treated animals. In another study, the effects of progressive dilutions (8 to 100% concentration) of representative fluids from estrogen- and progesterone-treated animals were studied in the fibrin plate system. Cellulose acetate electrophoresis of uterine fluid was performed in Owens buffer at constant

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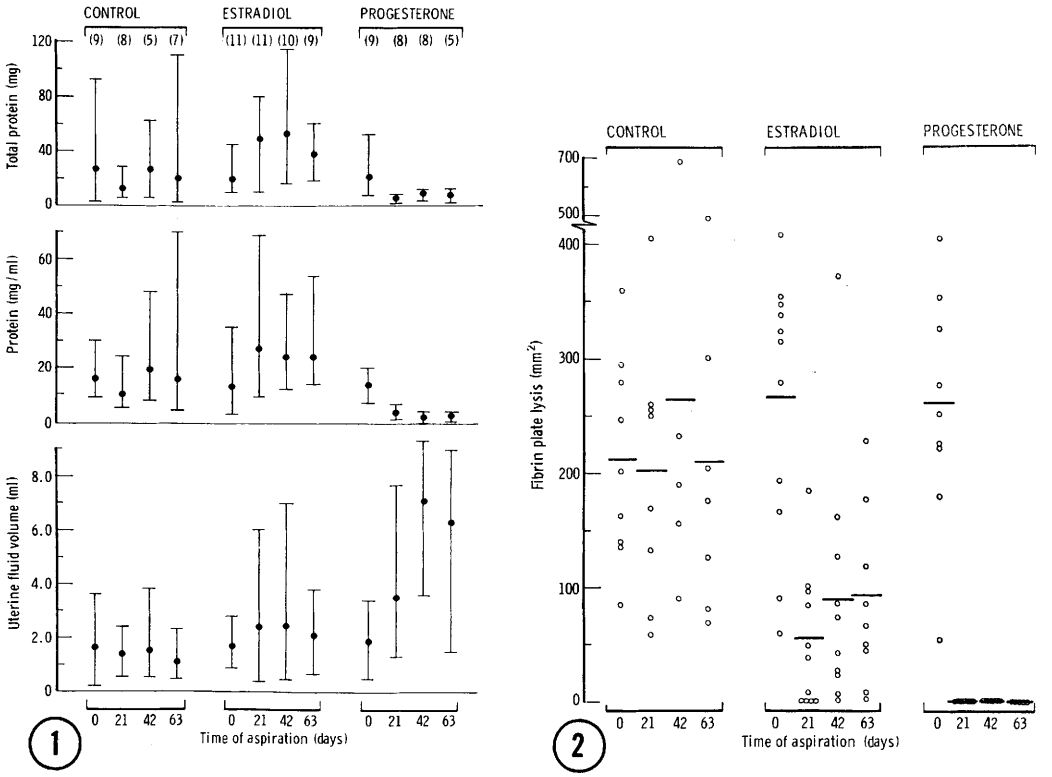

FIG. 1. Effect of estradiol and progesterone upon volume, protein concentration and total protein of mouse uterine fluid which is aspirated at each collection period. Figures in parentheses indicate number of samples assayed in each group. Mean and range of observations are indicated. There is no significant difference within the control group at any of the collection periods or between controls and pretreatment values at day 0 in the hormone groups. The volume increased in progesterone-treated animals (bottom graph), as compared to day 0, is significant at day 42 ($p < .001$) and day 63 ($.01 > p > .001$). Fall of protein concentration in the progesterone-treated group (middle graph) is significant at all collection periods ($p < .001$). Total protein content in estrogen-treated animals (top graph) increases as compared to day 0 at the 3 collection periods ($.01 > p > .001$), while total protein decreases in progesterone animals ($p < .001$).

FIG. 2. Effect of estrogen and progesterone upon bovine fibrin plate lysing activity of mouse uterine fluid. Open circles represent duplicate determinations on each individual sample, and horizontal lines indicate the mean. Fall in fibrinolytic activity in estrogen-treated group, as compared to day 0, is significant at day 21 ($p < .001$) and at days 42 and 63 ($.05 > p > .02$). Fall of fibrinolytic activity in progesterone group is significant at all collection periods ($p < .001$).

voltage (250 v) for 90 minutes. The cellulose acetate strips‡ were stained for protein by Procion brilliant blue M-RS and carbohydrates with the periodic acid-Schiff stain and analyzed on the Photovolt Densicord. The data were examined for properties consistent with the normal distribution. It was found that a log transformation gave the desired normal properties. The volume data required no change. After the necessary transformation the data were analyzed by a variance technique(12).

Results. Estradiol (Fig. 1) caused a slight but not significant rise in the volume of fluid harvested, as compared to the sesame oil controls. In progesterone-treated animals, as previously reported(8), the accumulated fluid increased 2- to 5-fold. The protein concentration per milliliter of uterine fluid increased nonsignificantly in the estrogen-treated animals, whereas it decreased significantly with the progesterone-treated animals.

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The total protein content of the fluid from estradiol-treated mice rose significantly, while there was a significant fall in the the progesterone-treated animals. Carbohydrate concentration varied widely in the estrogen-treated group and did not differ from the controls. In the progesterone-treated group the carbohydrate concentration paralleled the protein concentration.

Fibrin plate lysing activity (Fig. 2) was significantly suppressed in animals treated with estradiol as well as in those treated with progesterone. Plasminogen activator activity in the controls was unaffected by the experimental procedures. Comparison of pretreatment activity in each mouse with its own fibrinolytic activity during hormonal administration showed a decrease in every animal. Although fibrin plate lysis varied widely in the estrogen-treated animals at days 42 and 63, suppression of fibrin plate lysis to below 50 square millimeters occurred in 50% of these animals. Such low values did not occur in the control group. Decreased uterine fluid fibrinolytic activity and protein concentration were found in 3 of the progesterone-treated animals at day 21, before any increase in the volume of accumulated fluid occurred.

Serial dilution of samples of uterine fluid from estrogen- or progesterone-treated mice with low fibrin plate lysing activity caused a progressive concentration-dependent reduction of fibrinolysis. Progressive concentrations of a partially purified plasminogen activator had similar dose-dependent effects when saline, heated native uterine fluid, or fluid from estrogen-treated animals were used in constant concentrations.

The electrophoretic patterns of mouse uterine fluid obtained from estrogen-treated animals showed a general increase in protein in parallel with determinations by the Lowry method (Fig. 3). There was also a marked increase of periodic acid-Schiff staining carbohydrate in the estrogen-treated animals.

Discussion. Our studies show that administration of estrogen or progesterone suppresses fibrinolytic activity of the endometrial fluid of mice. As plasminogen-free fibrinogen and fibrin are not digested by mouse uterine fluid, its fibrinolytic activity assayed on plasminogen-rich fibrin plates is due to a plasminogen activator.

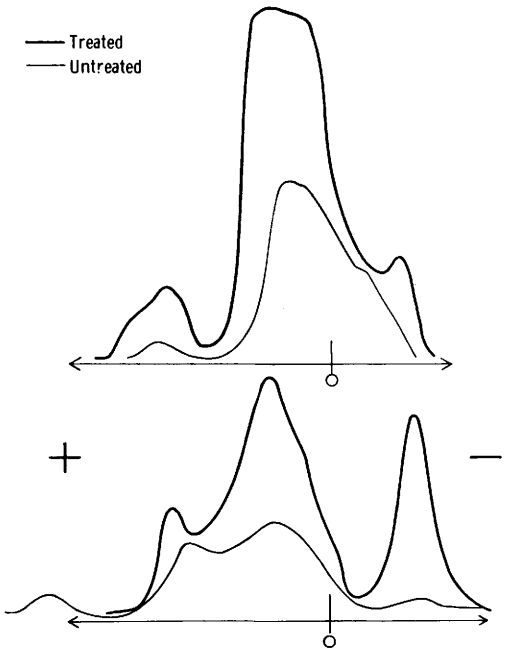

FIG. 3. Electrophoretic patterns of endometrial fluid from one representative mouse taken before medication and on 21st day of estrogen therapy and stained for proteins with Procion blue (bottom 2 tracings) and for carbohydrates with PAS (top 2 tracings). Origin of the electrophoretic migration is at 0. Protein concentration before treatment was 11.3 mg/ml, and on 21st day of estrogen treatment it was 18.3 mg/ml. Carbohydrate content before treatment was 3.6 mg/ml and on 21st day of estrogen treatment, 4.7 mg/ml. Fibrinolytic pretreatment activity was 324 mm^2, and on 21st day of estrogen treatment it was 0.

No inhibitors of plasminogen activation or of plasmin were induced by estrogen or progesterone administration. The serial dilution studies and use of partially purified plasminogen activator prepared from mouse endometrial fluid failed to demonstrate the presence of such inhibitory substances.

Dilution alone did not explain the reduced protein concentration and plasminogen activator activity in the endometrial fluid from progesterone-treated mice. This is suggested by disproportionately reduced total protein content in the progesterone-treated group and by loss of activator activity at a time when the fluid volume had not changed. The increase of protein in endometrial fluid of estrogen-treated animals reflects the anabolic

response of uterine tissues to estrogens. In contrast is the marked reduction of plasminogen activator caused by this hormone.

This unexplained suppressive effect of female sex hormones on plasminogen activator may not be limited to the endometrial fluid of mice. Thus, for example, estrogens suppress blood fibrinolytic activity in castrated rats(13). In human pregnancy, blood plasminogen activator decreases as hormones rise, and returns to normal after placental delivery(14,15).

Sex hormones may, therefore, suppress blood fibrinolytic activator activity similarly to the suppression demonstrated in uterine fluid of the mouse by both estrogen and progesterone.

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Effect of Pretreatment with Aggregate Albumin on Reticuloendothelial System Activity and Survival After Experimental Shock.* (31358)

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There is considerable evidence from studies of adaptive protection against shock to indicate that a direct functional relationship exists between the level of reticuloendothelial system (RES) activity and the tolerance of laboratory animals to various types of experimental shock(1,2,3,4,5). However most of the methods used to induce adaptation or resistance to shock *via* RES stimulation are not compatible for potential use in patients; *i.e.*, repeated trauma, saccharated iron oxide, endotoxins, thorium oxide, highly denatured serum proteins, etc. In these protection studies RES function was measured experimentally by its phagocytic capacity to clear the peripheral blood of known amounts of injected colloids.

Our laboratory has been exploring materials(6) which, from reported experience, should be safe to use in man and which could be expected to stimulate the RES to determine whether such stimulation did, in fact, occur and whether this also influenced survival after various types of experimental shock. The data reported here cover preliminary observations with a mildly denatured human serum albumin developed for use with an

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