Inhibitory Effect of Placenta on Plasminogen Activation in Human Organ Culture[1] (36376)

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During pregnancy, both the spontaneous fibrinolytic activity (1–6) and the response of the fibrinolytic activity to venous occlusion (7) successively decrease until term. After delivery, the fibrinolytic activity in the blood rapidly returns to nonpregnant level (1, 8, 9).

It has been shown that this decrease is dependent on the presence of the placenta and not of the fetus (10). It is, however, debatable whether the depression of the fibrinolytic activity should be ascribed to the high concentration of inhibitors in the placenta (11, 12) or to some effect of its hormonal secretion on the synthesis and release of activators of fibrinolysis (7, 8).

A recent method for studying the fibrinolytic activity in organ culture permits determination of the amount of fibrinolytic activators released from the explants. According to this method, explants are cultured in the presence of, but not in contact with, a preformed standard fibrin clot. Fibrinolytic activators released to the medium during culture cause a gradual breakdown of the clot. Determinations of fibrin degradation products (FDP) accumulating in the medium will give an indirect measure of the amount of fibrinolytic activators released.

Using this method we studied the inhibitory effect of human placenta on urokinase and on fibrinolytic activators released by human aorta and kidney explants. Fetal tissues were used because they can be cultured in a synthetic medium.

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Material and Methods. Aorta, kidneys, and placenta were taken from 18- to 24-week-old fetuses obtained at legal abortion of physically healthy women on sociomedical grounds. The fetuses were removed by hysterotomy.

The tissue fragments were washed in Parker 199 (SBL, Stockholm) culture medium and divided into pieces about 1 mm across. These explants were then placed on slices of gel foam (Spongostan, Ferrosan, Malmö), two explants per slice. The slices of gel foam with the explants were transferred to Leighton tubes (two slices in each tube) containing a clot formed by addition of 0.04 ml of thrombin (Topostasin, Roche) diluted to 75 NIH U/ml in Parker 199 to 1 ml of a solution of 1 g of fibrinogen, 80 ml of distilled water, and 20 ml of Parker 199. One milliliter of the culture medium, Parker 199, was then added. Care was taken to avoid contact between the sponge slices and the preformed clot.

In the experiments performed to assess the inhibitory effect of placenta on urokinase, placenta explants were cultured in medium containing respectively 3.0, 1.5, and 0.75 Ploug units of urokinase/ml (Green Cross). Each Leighton tube contained two slices of gel foam with three placenta explants on each. For comparison, the same test system was incubated with the same amounts of urokinase, but without placenta explants.

In the combined cultures two explants of placenta and two explants of aorta or kidney were placed on each slice of gel foam. Gel foam slices without explants placed in similarly prepared Leighton tubes served as controls.

For determination of FDP, 0.06 ml of cul-

TABLE I. Inhibition of Urokinase by Placenta.

Mean and range found for four culture tubes. Each value denotes FDP (mg/100 ml of Parker medium).

Days of culture	I	II	III
Urokinase, 3.0 U/ml	163 (75–225)	419 (400–425)	482 (425–550)
+ placenta	17 (6–22)	86 (75–90)	110 (90–125)
Urokinase, 1.5 U/ml	9 (6–14)	62 (39–75)	116 (50–160)
+ placenta	1.2 (0–4)	10 (2–25)	17 (6–37)
Urokinase, 0.75 U/ml	2 (0.8–3)	25 (9–54)	75 (40–140)
+ placenta	0	2.2 (1–3)	3.5 (2.5–4.5)
Placenta	0	0	1
Control	0	0	3

ture medium was collected every 24 hr with a capillary pipette. FDP were determined with a quantitative immunochemical method (13).

After culture, the explants were examined for fibrinolytic activity with Todd's fibrin slide method as modified by Pandolfi (14). Survival of the explants was checked by conventional histological examination.

Results. The inhibitory effect of placenta explants on the activation of plasminogen by urokinase is apparent from Table I, which shows a marked inhibition by urokinase in all the concentrations used. In the concentration of 0.75 Ploug units/ml of medium, the inhibition was almost complete.

There was a considerable release of fibrinolytic enzyme from the renal explants, as measured by the progressive increase of FDP in the culture medium. When the renal explants were cultured, together with placenta explants, the increase in FDP was much smaller (Table II).

Aorta explants released a moderate amount of fibrinolytic enzyme when cultured alone, but barely any when cultured together with placenta explants (Table III).

In the controls and in cultures of placenta alone, the amounts of FDP on the third day were negligible. Histochemical examination of the explants with the fibrin slide method at the end of the culture period revealed persisting activity in the aorta and kidney explants. Conventional histological examination

TABLE II. Inhibitory Effect of Placenta on the Fibrinolytic Activity in Kidney Organ Cultures.

Mean and range of determinations from four cultures. Each value denotes FDP (mg/100 ml of Parker medium).

Days of culture	I	II	III
Kidney	58 (30–86)	361 (225–520)	570 (480–700)
Kidney + placenta	2.5 (0–5)	31 (8–51)	75 (70–100)
Placenta	0	0	0.5
Control	0	0	2.5

TABLE III. Inhibitory Effect of Placenta on the Fibrinolytic Activity in Aorta Organ Cultures.

Mean and range of determinations from four cultures. Each value denotes FDP (mg/100 ml of Parker medium).

Days of culture	I	II	III
Aorta	2 (0.5–4.5)	8.5 (6–10.5)	54 (32–74)
Aorta + placenta	0	0	0.8
Placenta	0	0	0.5
Control	0	0	1.5

showed good survival of the explants.

An additional experiment was performed in which placenta and lung tissues were first cultured as organ culture for 2 days in Parker medium without a preformed clot. The medium from these cultures was then used as medium for aorta cultures in Leighton tubes with a preformed clot and also examined for its ability to inhibit urokinase. We found that medium from placenta cultures, but not from lung cultures, inhibited activation of plasminogen by urokinase and by the enzymes liberated from aorta explants.

Discussion. The results show that human placenta in organ culture liberates agents capable of inhibiting the activation of plasminogen by urokinase, as well as by fibrinolytic activators released by explants from kidney and aorta. That this inhibitory effect of placenta cannot be ascribed to adsorption of the activators to the surface of the placenta explants is apparent from the additional experiment, in which the medium from placenta culture had an inhibitory effect, while that from lung tissue had not. Persisting fibrinolytic activity in the aorta and kidney explants, as demonstrated histochemically, indicates that the inhibitory action takes place in the medium.

The progressive depression of fibrinolytic activity during pregnancy has been ascribed to a hormonal effect of the placenta on the synthesis and release of fibrinolytic activators from the vessel walls (7, 8). The demonstrated reduction of the fibrinolytic activity in combined cultures of placenta and kidney or aorta might be due to hormones secreted by the placenta explants influencing the synthesis and release of fibrinolytic activators from kidney and aorta explants. But the depressive effect of placenta explants on the activation of plasminogen by urokinase requires another explanation.

A connection has also been assumed between the inhibitors of urokinase found in the placenta and the reduction of the fibrinolytic activity during pregnancy (11, 12). Our finding that placenta can release inhibitors of fibrinolysis induced by urokinase lends support to this assumption. But urokinase has not been shown to be identical with the activator responsible for the fibrinolytic activity in the blood (15), whose level is believed to be maintained by a continuous release of activators from the vessel walls (16). Our observations show that placenta in the combined organ cultures liberates inhibitors acting also upon the activators released by vessel explants, suggesting a similar effect on the fibrinolytic activity *in vivo*.

Summary. The inhibitory effect of human placenta on plasminogen activation was studied with a method permitting determination of the fibrinolytic activity developed in organ culture. Placenta was found to liberate agents capable of inhibiting the activation of plasminogen by urokinase, as well as fibrinolytic activators released by explants from kidney and aorta. This inhibition may help to explain the well-known decrease of the fibrinolytic activity during pregnancy.

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