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Release of plasminogen activator from normal and neoplastic endometrium[1]

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Summary. In the medium of endometrial carcinoma cultures, anti-urokinase-reacting plasminogen activator was released in contrast to cultures of normal or hyperplastic endometrium.

It has long been known that neoplastic tissue in culture releases fibrinolytic enzymes. Certain transformed mammalian cells in culture release a plasminogen activator not released by their untransformed counterparts[2,3]. In human ovarian carcinoma, a stable plasminogen activator is released which has recently been shown to cross-react with the plasminogen activator present in urine, i.e. urokinase[4]. A radioimmunoassay (RIA) has been designed for urokinase and used for the detection of neoplastic anti-urokinase-reacting plasminogen activators[5].

We report here the release of a plasminogen activator from normal and neoplastic endometrium in culture determined by degradation of a fibrin clot preformed in the culture tube and by RIA.

Material and methods. Endometrial specimens were obtained at hysterectomy. Histological examination of the specimens was performed and the diagnoses were found to agree with those from previous curettage specimens.

The specimens were divided into pieces of about 1 mm^3 and cultured as organ cultures with 4 cultures per specimen on gelatin foam (Spongostan, Ferrosan) in Leighton tubes. A purely synthetic medium (Parker 199, SBL) was used supplemented with the tripeptide Gly-Lys-His[6]. The culture tube contained a preformed fibrin clot formed by adding 1 ml of human plasminogen containing fibrinogen (Fibrinogen Kabi, 1% in distilled water) to 0.02 ml of thrombin (Topostasin, Roche, 75 NIH U/ml saline)[7]. To some of the cultures, the inhibitor of plasminogen activation, tranexamic acid (AMCHA) was added to the medium in a concentration of 1-2 mg/ml. At 24-h-intervals after beginning of the culture, a small volume (0.06 ml) of medium was aspirated and assayed quantitatively for fibrin degradation products by immunoelectrophoresis[8] and for anti-urokinase-reacting material in the RIA. Survival of the explants was checked by histological examination at the termination of the culture period. Control tubes contained preformed clot, gelatin foam and medium.

For radioimmunoassay, an antiserum against human urokinase was produced in rabbits[9]. J^{125}-urokinase was prepared

Mean concentration of FDP and levels of anti-urokinase-reacting material in the culture medium of normal endometrium, adenomatous hyperplasia and endometrial carcinoma (n = number of patients)

	Mean value of FDP (μg/ml)			Mean levels of anti-urokinase-reacting material (ng/ml)
	Day I	Day II	Day III	Day III
Normal endometrium (n = 5)	466	2473	6121	4
Normal endometrium with AMCHA 0.01 mg/ml	0	10	1172	
Normal endometrium with AMCHA 0.02 mg/ml	0	10	1215	
Control	0	0	0	
Adenomatous hyperplasia (n = 3)	1757	4045	5481	4.2
Adenomatous hyperplasia with AMCHA 0.01 mg/ml	0	572	4750	
Adenomatous hyperplasia with AMCHA 0.02 mg/ml	0	584	4030	
Control	0	7	62	
Endometrial carcinoma (n = 7)	1725	5773	8203	53.3
Endometrial carcinoma with AMCHA 0.01 mg/ml	14	2318	4856	
Endometrial carcinoma with AMCHA 0.02 mg/ml	17	2269	4635	
Control	0	18	46	

with the lactoperoxidase method[10] and the RIA was performed with a double antibody system[5].

Results. The acticity of fibrinolytic enzymes released into the medium is given in the table, expressed in the concentration of FDP. The activity of fibrinolytic enzymes was low in cultures of normal endometrium, higher in cultures of hyperplastic endometrium and highest in the endometrial cancer cultures. Tranexamic acid in the concentrations used was not sufficient to inhibit completely fibrinolysis. In the medium of carcinoma cultures, anti-urokinase-reacting plasminogen activator was present in contrast to those of normal or hyperplastic endometrium.

Discussion. The results thus show that the amounts of fibrinolytic activators released were apparently related to malignancy. Urokinase-like plasminogen activator was found only in the culture of malignant endometrium. The traces of anti-urokinase-reacting material in the culture of normal and hyperplastic endometrium is probably due to unspecific precipitation. However, in neutralization experiments minor amounts of urokinase-like plasminogen activators have been detected in cultures of normal fetal lung and ureter cells[11].

The fibrinolytic activity in the cultures of benign or hyperplastic endometrium are probably due to release of the blood or tissue activator, which do not react in the assay[12]. The fibrinolytic inhibitor tranexamic acid did not completely prevent degradation of the clot in the concentrations used. In the same culture system tranexamic acid completely inhibited the fibrinolytic enzymes released from various embryonal organs including the fetal kidney[13]. The failure of tranexamic acid to prevent the degradation of the clot in the normal as well as malignant endometrial cultures, suggests the possible release of proteolytic enzymes other than plasminogen activators.

Production of stable plasminogen activator seem to be a property of the kidney and of certain malignant neoplasms[2-4]. Adenomatous hyperplasia is considered to be a premalignant state of the endometrium. Absence of urokinase-like enzymes in the cultures of hyperplastic endometrium and presence in those of endometrial cancer indicates a close relationship between the plasminogen activator synthesis and the genetic changes associated with malignant transformation.

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Fractionation of mouse alpha-fetoprotein[1]

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Summary. 4 distinct alpha-fetoprotein (AFP) containing fractions were obtained upon ion-exchange chromatography of late-gestational fetal mouse extracts. Despite this chromatographic heterogeneity, the individual AFP isolates were antigenically indistinguishable.

Mouse alpha-fetoprotein (AFP) exists in 5 electrophoretically distinct forms during fetal development[2,3]. This heterogeneity reflects differences in sialytransferase activity of yolk sac and fetal liver that produce modifications of the AFP molecule[4,5]. In this communication, the heterogeneity of mouse AFP by ion-exchange chromatography is demonstrated and the immunological properties of several AFP isolates are compared.

Materials and methods. Entire day 16-19 Swiss white fetal mice, dissected free of placentae and fetal membranes, were washed in phosphate-buffered saline (PBS) and homogenized in 0.14 M saline. The $5000 \times g$ supernatant was dialyzed against 10^{-3} M Tris, pH 7.2, and subjected to molecular sieving on 2.5×60 cm Sephadex G-100 and G-200 columns. The fractions in the 70,000 dalton region (determined by prior calibration with bovine albumin) of the G-100 elution profile were pooled, lyophilized, and reconstituted with 10^{-3} M Tris for separation on G-200. Small quantities (less than 20 mg) of G-200 Sephadex 70,000 dalton protein were fractionated on a 2.5×7 cm column of DEAE-Sephadex A-25[6,7]. Fractions were eluted with increasing concentrations of NaCl and their purity assayed by disc electrophoresis in 7% acrylamide[8]. Preparation of antisera to fetal mouse serum and AFP and immunoelectrophoretic procedures have been described[9]. Antisera to mouse transferrin and albumin (Cappel Laboratories, Cochranville, PA.) and anti-adult mouse serum were raised in rabbits.

Results and discussion. DEAE-Sephadex A-25 ion-exchange chromatography of the approximately 70,000 dalton 0.14 M saline-soluble fraction of fetal mouse homogenate yielded 9 peaks of 280 nm-absorbing material, the majority of which produced single bands in polyacrylamide gel. 2 transferrin-containing fractions, eluting at different NaCl concentrations were resolved that possessed distinct electrophoretic mobilities, confirming the reported microheterogeneity of this protein during murine development[2,3]. In contrast to the relative restriction of transferrin (fractions 3 and 4) and albumin (fractions 8 and 9) elution, AFP could be identified in peaks 5, 7, 8 and 9. Separate antisera prepared to fractions 5, 7, 8 and 9 were absorbed with equal volumes of adult mouse serum[9] and found to be specific for