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

Immunoelectrophoretic analysis* of mouse fetal antigens obtained by DEAE-sephadex A-25 chromatography

Fraction	Elution (M NaCl) from DEAE-Sephadex A-25	Mobility**	Anti-AFP	Anti-transferrin	Anti-albumin	Anti-fetal mouse serum	Anti-adult mouse serum
1	0	nd***	-	-	-	-	-
2	0	0.33	-	-	-	(1)β	(1)β
3	0.02	0.44	-	(1)β	-	(1)β	(1)β
4	0.05	0.50	-	(1)β	-	(1)β	(1)β
5	0.07	0.73	(1)α	-	-	(1)α	-
6	0.12	nd	-	-	-	-	-
7	0.15	0.78	(1)α	-	-	(1)α	-
8	0.18	0.78 and 1.00	(1)α	-	(1)alb	(2)α,alb	(1)alb
9	0.20	0.80 and 1.00	(1)α	-	(1)alb	(2)α,alb	(1)alb
Albumin	-	1.00	-	-	(1)alb	(1)alb	(1)alb

* The number of precipitin arcs resolved is given in parenthesis; the mobility of the antigen in agarose is appended thereto. ** Electrophoretic mobilities in 7% acrylamide gel, pH 9.0, relative to mouse albumin; when 2 bands were observed, the mobilities of each are given. *** Not detected in the gel.

AFP by immunoelectrophoresis. Ouchterlony analysis of individual day 14–19 fetal mouse extracts with each of these absorbed AFP-specific antisera yielded a single precipitin line of identity in all cases. Staining of the precipitin lines with amidoschwarz[10] also failed to disclose spur formation. Although chromatographically heterogeneous, each AFP species appeared antigenically indistinguishable. Murine AFP is known to exhibit considerable heterogeneity on ion-exchange and electrophoretic support media; AFP produced late in gestation is immunologically similar to AFP made earlier in development[11]. The fetal extracts used here would be expected to contain the 'immature'[4], less sialylated, forms of AFP in addition to the maximally sialylated protein and thus may contribute to the heterogeneity observed. Should post-translational addition of sialyl groups mask potentially unique antigenic determinants, the use of extracts containing just the maximally sialylated protein (day 18 fetal mouse plasma contains only the fully sialylated AFP[3]) as the test antigen may allow only for recognition of those antibody combining sites common to each of the several sialylated variants of AFP. Antigenic differences among fetal mouse AFP's could not be demonstrated, however, in extracts of fetuses as early as day 14 of gestation. This apparent conservation of antigenicity among heterogeneous AFP's contrasts with the well established existence of antigenically distinct variants or 'isoantigens' of carcinoembryonic antigen[12-14].

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Immunological profile of breast cancer patients in early or advanced disease[1]

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Summary. Immune reactivity of patients with early or advanced breast cancer, compared with healthy controls, has been measured using in vivo and in vitro tests. The results of our study show that impairment of cellular responsiveness occurred in women with advanced disease.

The data accumulated in the past few years suggest that impairment of immunologic reactivity occurs in breast cancer patients[4-6]. In the present report we describe our studies on the immune reactivity of breast cancer patients with early or advanced disease as compared with healthy controls.

The studies were performed on 34 women with histologically diagnosed breast cancer: 16 with early disease (average age 50, range 28–65) and 18 with advanced disease (average age 50, range 36–65). The 16 patients with early cancer included 10 at the 2nd stage of TNM classification (U.I.C.C.) and 6 at the 3rd stage. All immunological tests were performed prior to any treatment; all patients had a performance status (PS) higher than 40, according to the Karnofsky scale. Healthy controls were 36 volunteers of both sexes (average age 27, range 23–45). Immunological