COMPARISON OF INDIVIDUAL α-FETOPROTEINS OF HUMAN EMBRYOS AND PATIENTS WITH PRIMARY CARCINOMA OF THE LIVER

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Purified specimens of α-fetoproteins isolated from the sera of two human fetuses and three patients with primary carcinoma of the liver were compared by the agar diffusion, immunoelectrophoresis, and radial electrophoresis in polyacrylamide gel. The specimens were identical in their antigenic properties and differed only slightly in their electrophoretic mobility in agar and polyacrylamide gels.

α-Fetoproteins (α_f) are present in the sera of human and animal fetuses [2, 14, 15, 18]. The synthesis of this protein stops soon after birth, and no α_f can be found in the adult serum [3]. Synthesis of α_f is renewed when liver cells undergo malignant change, and this protein is found in the blood serum. This phenomenon was discovered in 1963 in mice [1], and later in other animals and man [4, 5, 11, 16].

The α_f of embryonic serum and α_f synthesized by tumors of the liver cannot be distinguished by the agar diffusion test [4, 10, 11]. However, this does not rule out the possibility of slight differences between the physicochemical properties or antigenic structure of α_f produced by the embryonic liver and that produced by liver tumors. To ascertain if such differences really exist, individual specimens of α_f from fetal sera and from individual patients with primary carcinoma of the liver must be compared in detail.

In the investigation described below a comparative analysis was made of individual purified specimens of α_f from fetal sera and from patients with primary carcinoma of the liver using immunochemical and physicochemical methods.

EXPERIMENTAL METHOD

The original material for obtaining the individual specimens consisted of sera of 2 human fetuses (14th and 24th weeks of pregnancy) and the sera of 3 patients with primary carcinoma of the liver kindly provided by Professor Masseive and Dr. Leblanc (University of Dakar). The specimens of α_f were obtained from 5 ml of each sample of serum by preparative electrophoresis in polyacrylamide gel as described previously [7, 8]. After each stage of purification the α_f eluates were concentrated at 4°C for 12-14 h with polyethylene glycol (M 40,000) at the rate of 1 g per 8 ml of fluid to be concentrated. The final specimen was dialyzed against distilled water for 2-3 days at 4°C and then lyophilized.

Pure specimens of individual α_f isolated from human fetal sera were designated α_{f14} and α_{f24}, and the corresponding specimens from the patients' sera α_{f224}, α_{f235}, and α_{f237}.

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Monospecific antisera were obtained by inoculating rabbits into a lymph gland with the pure specimen of α_f obtained from a mixture of sera of several human fetuses and with the specimen of α_{f235} [9].

The α_f specimens were compared by the agar diffusion test [6], and their relative electrophoretic mobilities were determined by immunoelectrophoresis in agar [12] and by analytical radial electrophoresis in polyacrylamide gel [8]. The zero point for determination of realtive mobility in agar was taken as the cathode edge of the γ-globulin arc of human serum, corresponding to the position of the pyronine after electrophoresis. The distance from the zero point to the centers of the antigen arcs was measured. The distance from the zero point to the center of the human albumin (D_A) arc was taken as unity. The relative mobility was defined as the ratio $D\alpha_f/D_A$. The distance $D\alpha_f$ from the upper part of the finely porous gel to the lower edge of the zone of the substance, and the ratio between $D\alpha_f$ of the patients and $D\alpha_f$ of the fetuses was determined.

EXPERIMENTAL RESULTS

The results of a test to compare individual specimens of α_f with the aid of antiserum against fetal α_f are shown in Fig. 1a. All the specimens were identical with each other and with the antigen of the test system. Their identity was also indicated by tests in which the same antiserum was exhausted by individual specimens of α_f. After exhaustion with specimens of both α_{f14} and α_{f224}, the antiserum no longer reacted with the antigen of the test system or with any of the individual α_f specimens (Fig. 1b).

It could be postulated that differences between the antigenic structure of the α_f specimens tested would be detected by antiserum against α_{f235}. However, no differences were found between them by the use of this antiserum also.

Comparison of the electrophoretic mobility of the individual antigens in agar in one particular test revealed slight differences. Specimens of fetal α_f had equal relative electrophoretic mobility, namely 0.95. The electrophoretic mobility of α_f from patients with carcinoma of the liver also was equal (0.88), but was 7% lower than the relative mobility of human fetal α_f (Fig. 2). Analogous results were obtained in the 2 tests carried out. The difference between the electrophoretic mobility possibly indicates a difference in the charge on the α_f molecule of the patients and that on the fetal α_f molecules.

With these results in mind, it was decided to compare the α_f specimens by radial electrophoresis in polyacrylamide gel, for in this method fractionation takes place in accordance with both charge and molecular weight of the substances.

The results of radioelectrophoresis (Fig. 3a-e) showed that the mobility of α_f specimens from patients with carcinoma of the liver was 0.96 if the fetal mobility was taken as 1. The very slight difference in length of the path of α_f from sera of the patients and fetuses was probably due to differences in the concentrations of these specimens. A mixture of all α_f specimens also migrated as a single homogeneous zone during electrophoresis in polyacrylamide gel (Fig. 3f). By this method, no significant differences

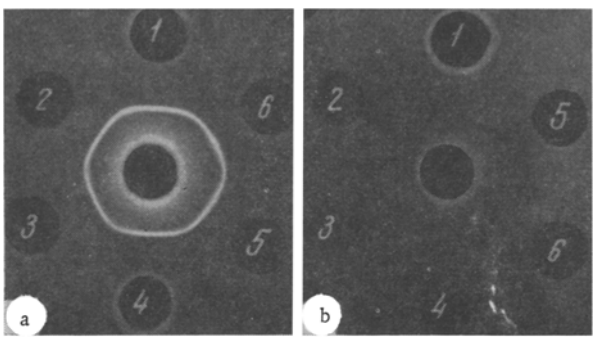

Fig. 1. Reaction of identity of individual α_f specimens. Central wells: a) antiserum against human embryonic α_f, b) same antiserum exhausted with α_{f14}; peripheral wells: 1, 4) human fetal serum (antigen of test system), 2, 3, 5, 6) α_{f235}, α_{f237}, α_{f14}, and α_{f24} respectively.

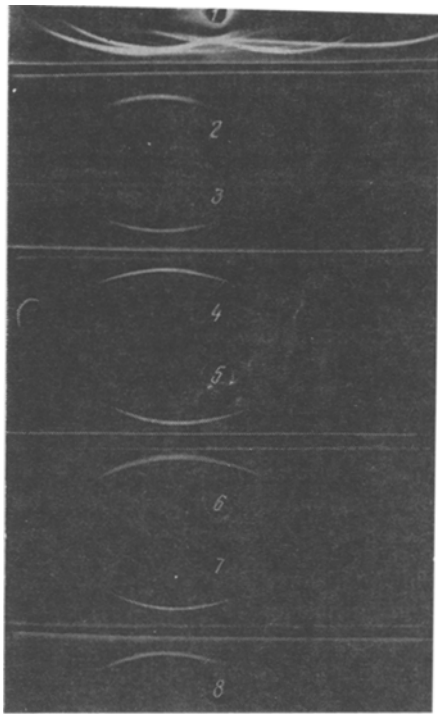

Fig. 2. Comparison of electrophoretic mobilities of individual α_f samples by immunoelectrophoresis. Wells contain: 1) serum of patient 235; 2, 3, 8) α_{f24}; 4, 5, 6, 7) α_{f14}, α_{f237}, α_{f235}, and α_{f224} respectively. Top gutter contains mixture of antisera against adult human serum proteins and human embryonic α_f (1:4). Remaining gutters contain antiserum against embryonic α_f.

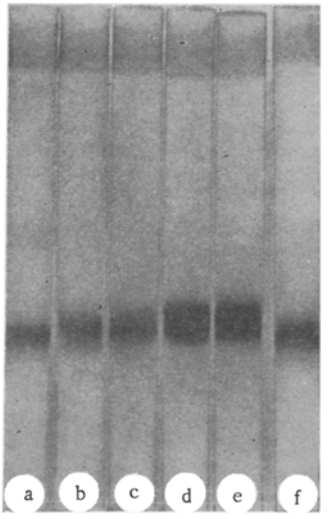

Fig. 3. Comparison of electrophoretic mobilities of individual α_f specimens by radial electrophoresis in polyacrylamide gel. a, b, c, d, e) α_{f24}, α_{f14}, α_{f224}, α_{f235}, and α_{f237} respectively; f) mixture of all these proteins.

were thus found in the electrophoretic mobilities of these proteins. The possibility is not ruled out that differences could be found if large numbers of individual α_f specimens were studied, for an electrophoretic form of "slow" α_f has been found in 1 of 150 tested sera of patients with primary carcinoma of the liver by electrophoresis in starch gel [17].

It can be concluded from these results that α_f from human fetuses and from patients with hepatomas are identical in their antigenic properties but differ in their electrophoretic mobility. The differences between the electrophoretic mobilities of these patients are more marked during electrophoresis in agar than by the use of polyacrylamide gel.

Determination of the amino-acid composition, the molecular weight, and other physicochemical properties of these substances is essential before final conclusions can be drawn regarding the identity or difference between α_f specimens synthesized under normal and pathological conditions.

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