DETERMINATION OF BACKGROUND SERUM ALPHA-FETOPROTEIN LEVEL IN BLOOD DONORS BY COMBINED ELECTROPHORESIS AND PRECIPITATION IN POLYACRYLAMIDE GEL

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Alpha-fetoprotein (AFP) was detected by combined electrophoresis and precipitation in polyacrylamide gel (EPAG) in the sera of healthy blood donors. AFP was found in about half of the sera in concentrations of not more than 3 ng/ml. AFP could not be reliably determined in the other cases, the results being at the limit of sensitivity of the method. EPAG thus gives results fully comparable in sensitivity with the radioimmunological test and it fully retains the resolving power of double diffusion in gel. EPAG can be used to verify the specificity of the results of the radioimmunological test.
KEY WORDS: *alpha-fetoprotein; electrophoresis-precipitation in polyacrylamide gel (EPAG).*

Alpha-fetoprotein (AFP), an embryonic serum protein, is present in the blood of the human embryo in a concentration of 3-4 mg/ml and in adult human blood in a concentration of not more than 10 ng/ml [1, 5-7]. An increase in the AFP level is characteristic of primary carcinoma of the liver, teratoblastomas of germinal origin, and certain nonneoplastic diseases of the liver [1, 7]. AFP can be detected in healthy adult human blood only by the most sensitive radioimmunological methods, with a sensitivity of the order of 1 ng/ml [5-7]. The specificity of these methods is completely determined by the degree of purification of the antigen, for by themselves the methods do not possess resolving power and they cannot differentiate between a particular antigen and others present as impurities. A highly sensitive radioimmunodiffusion method of determination of antigens was developed previously in the writers' laboratory, namely combined electrophoresis and precipitation in polyacrylamide gel (EPAG), capable of detecting AFP with a sensitivity of 0.5-1 ng/ml and with the resolving power of double diffusion in gel [2].

In the present investigation the presence of AFP in sera of normal blood donors and patients with various liver diseases of nonneoplastic nature was investigated by EPAG.

EXPERIMENTAL METHOD

Sera from male blood donors, obtained from the Moscow Blood Transfusion Station, and also sera from Abidjan (Ivory Coast) collected during an examination of the population of this region for the presence of AFP, were used. The sera were kept at 4° and −20°C. Sera from patients with various liver diseases obtained from Moscow clinics also were studied. Before determination, all the sera were dialyzed against 0.06 M Tris–HCl buffer, pH 6.7. The tests were carried out by the method described previously [2] with the following modifications.

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Fig. 1

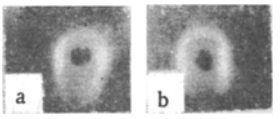

Fig. 2

Fig. 1. Calibration of human AFP in EPAG using autoradiography: a) 1.5, b) 0.7, c) 0.3 ng/ml. Test system 1/128.

Fig. 2. AFP in serum of healthy male blood donor: a) donor from Moscow (AFP concentration 3 ng/ml); b) donor from Africa (Ivory Coast). Test system 1/128.

1. A) The finely porous gel used consisted of 5.6% gel (Cyanogum 41, Serva, West Germany) in 0.38 M Tris–HCl buffer, pH 8.9 (Tris 4.6 g, conc. HCl 0.4 ml, H_2O to 100 ml). The large-pore concentrating gel consisted of 4% Cyanogum in 0.06 M Tris–HCl buffer, pH 6.7 (Tris 0.75 g, conc. HCl 0.5 ml, H_2O to 100 ml). The two gels were polymerized in the presence of TEMED and ammonium persulfate (1 mg/ml). The electrode buffer was a 0.012 M Tris–glycine buffer, pH 8.3 (Tris 15 g, glycine 145 g, H_2O to 1 liter).

B) The chamber contained four reservoirs for antigen (15 × 30 mm). The samples for analysis consisted of 0.25 ml undiluted serum, which was mixed with an equal volume of 8% Cyanogum in Tris–HCl buffer, pH 6.7.

2. The gel plates were washed to remove protein, labeled with I^{125}-anti-γ-globulin, and washed to remove labeled antibodies in a vessel as described previously [3]. Two strips of gel were placed in the vessel through which 10 liters of 0.14 M NaCl solution was passed for 15-18 h. Under slight pressure, a solution of I^{125}-antibodies (0.6 mCi/mg, 0.013 mg/ml) was introduced into the vessel for 1 h at room temperature and overnight at 4°C.

After incubation the labeled antibodies were drawn off *in vacuo* and the vessel connected for 15-18 h to a 10-liter bottle containing physiological saline for washing.

The washed strips of gel were covered with liquefied 1% agar and, after drying, they were exposed on RF-3 film for between 8 and 48 h.

3. Antigen and antiserum of the"immunodiagnosticum for human hepatocellular carcinoma and teratoblastomas" prepared by the N. F. Gamaleya Institute of Epidemiology and Microbiology were used as the test system. The AFP concentration in the antigen of the diagnosticum was 45-50 μg/ml. The test serum was diluted 4 times for the reaction in the visible zone and 128-256 times for the autoradiographic study.

As the standard AFP, a preparation of international standard AFP, batch 72/225, containing 50 international units (i.u.) AFP/ml, equivalent to about 100 μg/ml [8], was used. The standard was obtained from the International Agency for the Study of Cancer. Twofold dilutions of the standard were prepared in Tris–HCl buffer, pH 6.7, containing 1/400 bovine serum.

EXPERIMENTAL RESULTS

Calibration. For tests with the international standard AFP taken for calibration and as the standard substance, AFP in the visible region was determined to a concentration of 45-90 ng/ml, and by the use of autoradiography to a concentration of 0.7-1.5 ng/ml (0.36-0.7 i.u.) (Fig. 1).

AFP in Donors' Sera. All the sera were first tested by indirect immunoradiography [4] and did not contain AFP (15-30 ng/ml). Of 200 sera tested, AFP was clearly, regularly, in repeated tests, and in a concentration of not more than 3 ng/ml in about half of the cases (Fig. 2a). In the other sera AFP was found irregularly, at the limit of sensitivity of the method. AFP was detected more clearly in sera from the African donors, where it evidently existed in a higher concentration than in Europeans (Fig. 2b). Strongly positive results were obtained in 28 of 50 cases.

AFP was found in all sera from the 20 patients with cirrhosis of the liver (patients from Moscow clinics), even though they were negative in the radioimmunodiffusion test.

In this investigation the presence of a background level of AFP in the blood of healthy blood donors was thus demonstrated by a direct method. The results indicate that EPAG and the radioimmunological test are fully comparable in sensitivity. The writers consider that EPAG can be used to verify the specificity of results obtained by the radioimmunological method.

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