TWO COMPONENTS OF HUMAN α-FETOPROTEIN DETECTED BY ELECTROFOCUSING

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An α-fetoprotein was isolated from the ascites fluid of a patient with primary carcinoma of the liver, at first by electrophoresis in a sucrose density gradient or in polyacrylamide gel, and later by isoelectric focusing in a pH gradient of 4.0-6.0 and 4.5-5.5. The degree of purification achieved by a combination of these methods is lower than by the immunological method. During isoelectric focusing the α-fetoprotein is divided into two fractions with isoelectric points of 4.78±0.01 and 5.20±0.01 respectively. The ratio between these fractions is about 4:1.

Experiments [1] have shown that the detection of α-fetoprotein (AFP) in blood serum can be used as a diagnostic test for primary carcinoma of the liver. It was found later [8] that another embryo-specific antigen appears in the blood serum of patients with carcinoma of the rectum and colon and that it is characteristic of these types of malignant tumor, so that a method of diagnosis of human intestinal carcinoma has been developed on this basis. The specific embryonic antigen also appears on the surface of malignant cells in other malignant tumors, such as tumors of the human stomach [7]. This fact has stimulated interest in the more detailed study of these embryonic proteins.

The object of this investigation was to discover whether isoelectric focusing can be used to obtain an immunologically pure preparation of AFP from the ascites fluid of patients with primary carcinoma of the liver, containing this protein [2].

EXPERIMENTAL METHOD AND RESULTS

The ascites fluid was lyophilized and stored at 4°C until required for use. Usually 1 g of the lyophilized preparation was used in the experiment and was dissolved in 7 ml standard tris-EDTA-borate buffer, pH 8.9 [6], diluted 1:5 with distilled water. The resulting solution was dialyzed against the same buffer at 4°C for 12 h and then centrifuged at 40,000 g for 30 min. Residue was discarded and the total protein concentration determined in the solution refractometrically and spectrophotometrically (from the extinction at 280 nm). The concentration of AFP was determined from the titer of the gel diffusion test using antiserum obtained as described previously [2]. The solution containing AFP was fractionated initially either by electrophoresis in polyacrylamide gel, as described in [2, 3], or by electrophoresis in a sucrose density gradient on an LKB 3340 column. The sucrose density gradient was formed with the aid of the LKB 8122 gradient mixer. The column was loaded with 3.5 ml of 6.75% protein solution. The method of electrophoresis was that described in the instructions with the apparatus.

The results are shown in Fig. 1a. During electrophoresis in a sucrose density gradient at pH 8.9 the AFP was located chiefly in the zone of α_1-globulin. The AFP was located in the corresponding zone during electrophoresis in polyacrylamide gel [2, 3]. Fractions containing AFP (from 45 to 90 ml) were

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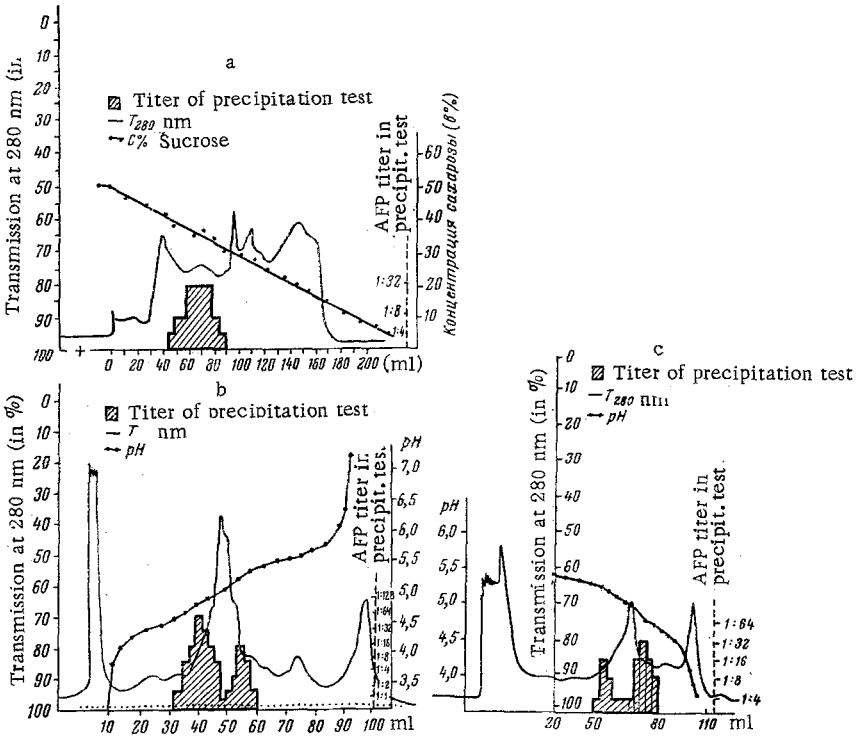

Fig. 1. Fractionation and purification of human α-fetoprotein by electrophoresis and by isoelectric focusing: a) electrophoresis in sucrose density gradient (Y = 800 V, I = 5 mA, T = 4 h); b) isoelectric focusing in pH gradient 4.0-6.0 (Y = 1000 V, I = 1.05 mA, T = 72 h); c) isoelectric focusing in pH gradient 4.4-5.5 (Y = 1100 V, I = 0.9 mA, T = 168 h).

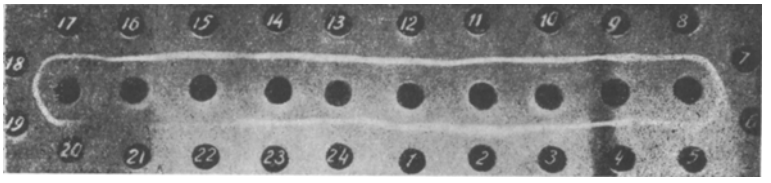

Fig. 2. Ouchterlony's immunodiffusion in gel. Middle row: antiserum against human AFP; 11, 18, and 24) test system AFP; 22) original AFP; 23) AFP after electrophoresis; 1-10) AFP-1 in various dilutions to 1:16; 12-17, 19) AFP-2 in various dilutions to 1:16.

pooled and dialyzed against deionized water until the conductance of the solution had fallen at least to 50 μmoles. The conductance was measured by means of a type SDM-2 (Radiometer, Denmark) conductometer. Isoelectric focusing of the solution was carried out in an LKB 8110 column. The pH 4.0-6.0 ampholin was used. Isoelectric focusing was carried out according to the instructions given with the apparatus. The results of a typical experiment are shown in Fig. 1b.

Clearly during isoelectric focusing the AFP was divided into two components with different isoelectric points. An increase in the duration of isoelectric focusing up to 9 days did not alter the result.

To purify the AFP-1 and AFP-2 further, isoelectric focusing was repeated within a narrower zone of pH (4.5-5.5) in accordance with method B described in the instructions to the apparatus. The results are shown in Fig. 1c. During repeated isoelectric focusing both the AFP-1 and the AFP-2 were again separated into two components, and the ratio of AFP-1 : AFP-2, just as after the first isoelectric focusing, was also approximately 4 : 1.

TABLE 1. Analysis of Fractions Obtained by Purification of AFP

Material	Total protein (in mg)	AFP (in μg)	pH_i (±0.01)	Total protein of AFP
Ascites fluid	490	1800		280
Fraction containing AFP obtained by electrophoresis in sucrose gradient	110	1800		50
Isoelectric focusing in pH gradient 4.0-6.0:				
Taken in experiment	98	1780		50
Fraction 1 (AFP-1)	29,5	1280	4,78	20
Fraction 2 (AFP-2)	24,0	320	5,20	8
Isoelectric focusing in pH gradient 4.5-5.5				
Taken in experiment AFP-1	10,0	640	4,78	20
Obtained:				
AFP-1	2,3	436	4,78	16
AFP-2	1,2	124	5,20	10
Taken in experiment AFP-2	13,0	300	5,20	10
Obtained:				
AFP-1	3,1	220	4,78	15
AFP-2	1,1	64	5,20	2

Content of AFP in corresponding fractions calculated from titer of precipitation test, bearing in mind the known sensitivity of this test [4].

The result obtained by testing the immunological identity of AFP-1 and AFP-2 by the gel diffusion test is shown in Fig. 2. Immunologically it is evident that AFP-1 is identical with AFP-2, and also with the original preparation of total AFP.

The results of quantitative analysis of the fractions obtained by purification of the AFP in accordance with the procedure described are given in Table 1.

The results given in Table 1 show that it is impossible by means of a combination of isoelectric focusing and electrophoresis to achieve the same degree of purification of AFP as by the other methods used previously [2, 3]. However, in the course of this investigation it was discovered that the AFP consists of two components. The AFP is evidently a protein which exists in two forms in equilibrium with each other (perhaps a dimer and monomer).*

While this paper was being prepared for publication, a short communication from Alpert et al. appeared [5]. These workers also found that human AFP consists of two components. According to their observations pH_i for AFP-1 is 4.78 ± 0.05. No value is given by them for pH_i for AFP-2.

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