COMPARATIVE STUDY OF FETUIN AND EMBRYO-SPECIFIC α-GLOBULIN IN BOVINE FETAL SERUM

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Fetuin and embryo-specific α-globulin differ in several of their physicochemical and antigenic properties. They can therefore be regarded as different embryonic proteins.

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The object of this investigation was to study some of the physicochemical and antigenic properties of fetuin and embryo-specific α-globulin (ESA-globulin) in bovine fetal serum [11].

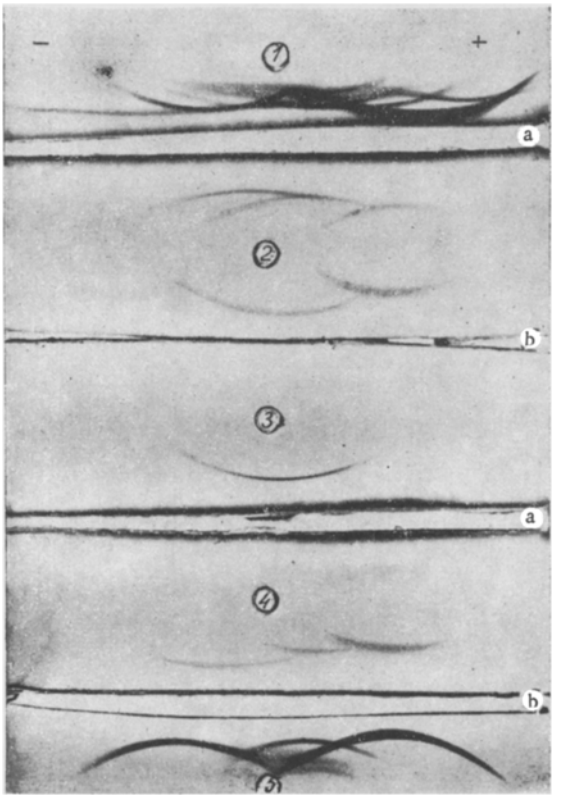

Fig. 1. Immunoelectrophoretic investigation of preparations of bovine fetuin and fetal ESA-globulin. 1) Blood serum of adult cow; 2) fetuin isolated by Deutsch's method; 3) ESA-globulin, pure; 4) fetuin isolated by Spiro's method; 5) bovine fetal blood serum; a) antiserum against bovine fetal serum proteins; b) antiserum against adult bovine serum proteins.

EXPERIMENTAL METHOD

The physicochemical properties of fetuin and ESA-globulin were compared by several methods of fractionation of fetal sera (with ammonium sulfate, by Cohn's X-method [1]) followed by immunochemical analysis of the separated fractions, and also by studying isolated preparations of these proteins. Characteristic reactions were carried out with the isolated proteins to test for the presence of lipid and carbohydrate components, by staining with Sudan black and by the periodic acid-Schiff (PAS) method after electrophoresis in agar gel [6].

Fetuin was isolated by the methods of Deutsch [3] and Spiro [12]. ESA-globulin was isolated immunochemically from the specific precipitate. Antiserum against ESA-globulin was obtained by immunizing rabbits with pooled blood serum from bovine fetuses, followed by exhaustion of the serum and verification of its monospecificity. Bovine fetal serum was added in a volume of 10 ml to 200 ml of monospecific antiserum, and the precipitate was sedimented and compacted in a centrifuge after adjustment of the pH to 8.0. Reprecipitation in this way was repeated three times. The precipitate was then suspended in 100 ml 1% alcoholic solution of TCA and allowed to stand for 1 h at 4°, with shaking from time to time, after which it was centrifuged at 6000 rpm for 15 min. The supernatant was dialyzed in cellophane bags for 48 h in tap water, and further concentrated by dialysis against 20% polyvinylpyrrolidone solution. The purity of the preparation was tested immunochemically and by disc electrophoresis in polyacrylamine gel.

The immunochemical investigation was carried out by Ouchterlony's method [9], and by the method of immunoelectrophoresis described by Grabar and Williams

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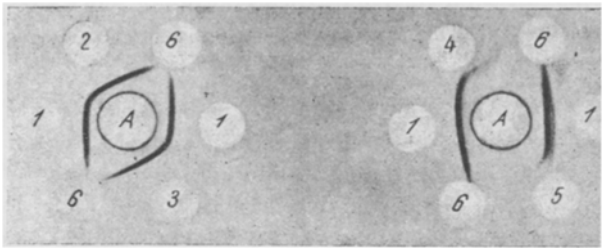

Fig. 2. Immunodiffusion analysis of isolated proteins for the presence of ESA-globulin. 1) Bovine fetal blood serum diluted 1 : 10; 2) 0.5% solution of ESA-globulin diluted 1 : 40; 3) 0.5% solution of fetuin isolated by Spiro's method; 4) 1.5% solution of fetuin isolated by Deutsch's method; 5) 0.5% solution of ESA-globulin diluted 1 : 1000; A) antiserum against ESA-globulin, monospecific; 6) physiological saline.

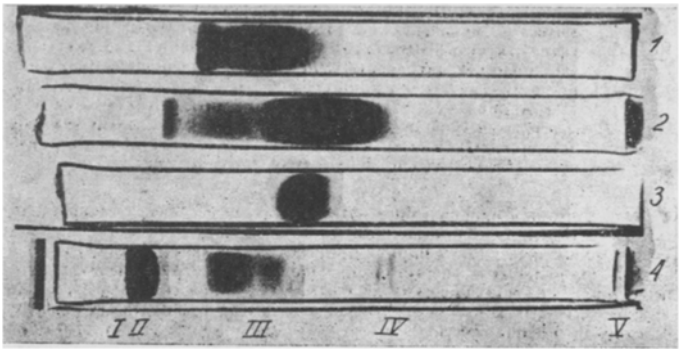

Fig. 3. Electrophoresis of isolated proteins obtained by the disc method. 1) Fetuin isolated by Deutsch's method; 2) ESA-globulin, pure; 3) fetuin isolated by Spiro's method; 4) bovine fetal blood serum; I) prealbumin; II) albumin; III, IV, and V) zones of postalbumins, transferrin, and macroglobulins respectively.

[5]. Two antisera were used for the immunochemical analysis: antiserum against bovine serum proteins prepared in the Gamaleya Institute of Epidemiology and Microbiology (batch 2), and antiserum against bovine fetal serum proteins, prepared by the author, both whole and exhausted.

The fetal sera and the isolated protein preparations were investigated by disc electrophoresis in 7.5% polyacrylamide gel [2, 8].

EXPERIMENTAL RESULTS

During fractionation of fetal sera with ammonium sulfate at room temperature, fetuin begins to be precipitated at 33% saturation and it is completely precipitated by a semisaturated solution of ammonium sulfate [3, 4], whereas ESA-globulin begins to be precipitated at 43% saturation and is completely precipitated by 65% saturation with ammonium sulfate. These results agree with those obtained by Masopust [7] working with human ESA-globuin. During alcoholic precipitation of calf fetal sera by Cohn's X-method [1], most ESA-globulin was precipitated in fractions IV and V, whereas fetuin was left in fraction VI, from which it was isolated by further purification [12].

ESA-globulin was readily precipitated by a neutralized 5% solution of TCA, in which fetuin is readily soluble. The fact that fetuin is precipitated by ammonium sulfate and is soluble in 5% TCA solution is the basis of its separation by Deutsch's method [3].

When preparations of isolated proteins were stained with Sudan black after electrophoretic fractionation in agar gel, the preparations of fetuin and ESA-globulin were not stained, although in bovine fetal serum a considerable lipoprotein fraction was revealed in the zone of the α-globulins.

Both preparations of fetuin took up the dye readily when testing for glycoproteins by the PAS method. Whereas ESA-globulin did not stain, indicating that it contained no carbohydrates or only very small traces – less than 1.5% [10]. Immunochemical investigation of the isolated preparation of ESA-globulin showed it to be homogeneous both in Ouchterlony's precipitation reaction and in immunoelectrophoresis (Fig. 1). This protein had the mobility of α-globulin.

Immunoelectrophoretic analysis of the fetuin preparation isolated by Spiro's method revealed three components, compared with 5 antigenic components in the fetuin isolated by Deutsch's method.

Ouchterlony's precipitation reaction performed with titration of antigen demonstrated that a 0.5% solution of fetuin isolated by Spiro's method contains only about 1/40 of the ESA-globulin present in a 0.5% solution of the ESA-globulin prepared by the author, while fetuin isolated by Deutsch's method (concentration 1.5%) contained only 1/1000 of that present in the 0.5% ESA-globulin solution (Fig. 2). Simple calculations show that fetuin isolated by Spiro's method contains about 2.5% of ESA-globulin, while fetuin isolated by Deutsch's method contains only about 0.03% of ESA-globulin.

It has not yet proved possible to obtain antisera revealing any embro-specific proteins other than ESA-globulin by immunization of rabbits and hens with fetuin preparations, and this is possible evidence of the weak antigenicity of fetuin.

A study of the isolated preparations and of whole calf fetal blood serum by disc electrophoresis in polyacrylamide gel yielded the following results. Isolated ESA-globulin is homogeneous and migrates with the mobility of the slow postalbumins. A considerable albumin fraction was found in feutin isolated by Deutsch's method, along with a massive fraction of fast postalbumin. Besides a massive fraction of slow postalbumin, the preparation of fetuin isolated by Spiro's method also contained small traces of prealbumin, albumin, fast postalbumin, and a protein migrating somewhat more slowly than the main fraction. The difference in the mobility of fetuins isolated by the different methods is noteworthy (Fig. 3). The following fractions could be distinguished in whole bovine fetal blood serum by disc electrophoresis: prealbumin, albumin, followed by 4-5 fractions of postalbumins, not completely identified, after which from 3 to 5 fractions migrated in the transferrin zone, and last of all, α-macroglobulin, hardly able to penetrate into the microporous gel (Fig. 3).

The results thus demonstrate differences in several physicochemical and antigenic properties of the fetuin and ESA-globulin of bovine fetuses. They suggest that fetunin and ESA-globulin are different embryonic proteins.

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