THE DEVELOPMENT OF SIMILAR ANTIGENIC DETERMINANTS IN EMBRYOSPECIFIC α-GLOBULINS OF MAN AND CERTAIN ANIMALS

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It was shown [4] that antiserum to bovine fetuin used for immunomanifestation of human fetal serum gives a cross precipitin reaction in the α-globulins zone. Subsequently it turned out that the crystalline bovine fetuin was insufficiently pure, since antisera to it reacts not only with bovine, goat and sheep fetuin but also with certain fractions of the serum proteins of these same adult animals [3]. Therefore it was not possible to consider antifetuin serum monospecific while the previous results [4] are sufficiently convincing.

The purpose of the present work was to determine to what extent the embryonic α-globulins of man, dog, bull, sheep, pig, rabbit, guinea pig and rat are species specific. For comparison of the immunochemical analysis we used rabbit antisera which reacted only with fetal serum proteins with the absence of a noticeable precipitation reaction with any components of the serum of adult individuals.

METHOD OF INVESTIGATION

Antisera to embryospecific proteins was obtained by immunizing rabbits with sera of fetuses of different stages of intrauterine development. One to two months after the first immunization cycle reimmunization was carried out by means of a single injection of the appropriate fetal serum (500-100 mg protein). Seven to ten days after reimmunization blood was taken and, as a rule, hyperimmune serum obtained. For the experiments only antisera which after depletion with an excess of adult serum continued to precipitate fetal α-globulins was selected. In the case of a small amount of antibodies to embryospecific α-globulin repeated reimmunizations (from 3 to 5 times) were carried out after 1-3 months.

The followings antisera against fetal serum were used in the work: 1) human—anti-human fetal sera (No. 15 and 104), 2) cow—anti-fetal cow sera (No. 118 and 119), 3) dog—anti-fetal dog sera (No. 100 and 134), 4) pig—anti-fetal pig sera (No. 86), 5) rat—anti-newborn sera (No. 38 and 787).*

The fetal sera of man (SPCh), dog (SPS), bull (SPB), pig (SPSv), sheep (SPBar), rat (SPRat) and newborn rabbit (SNK) and guinea pig (SNMs) were used as antigens. Normal sera of adult animals and humans were used to deplete the corresponding antisera and for control of cross precipitation reactions.

Immunoelectrophoresis was carried out in an EFA-1 cell specially adapted by us for semimicroanalysis according to Grabar and Williams [5]. The technique of the work and the experimental conditions were described earlier [2]. To study cross serological reactions among embryospecific components the method of double diffusion in agar in the usual set-up according to Ouchterlony [7] and also in the modification of G. I. Abelev [1] was used.

We determined the electrophoretic mobility of each embryospecific globulin relative to the electrophoretic mobility of the serum proteins of an adult human according to the data of immunoelectrophoresis in agar (Fig. 1).

As the immunoelectrophoretic analysis showed the embryospecific components of bull and sheep fetal serum possess similar motility and could be related to α_2-globulins, while the fetal components of the serum of fetal pigs,

*Antisera against the serum of newborn rats was kindly given to us by G. I. Abelev to whom we extend our sincere gratitude.

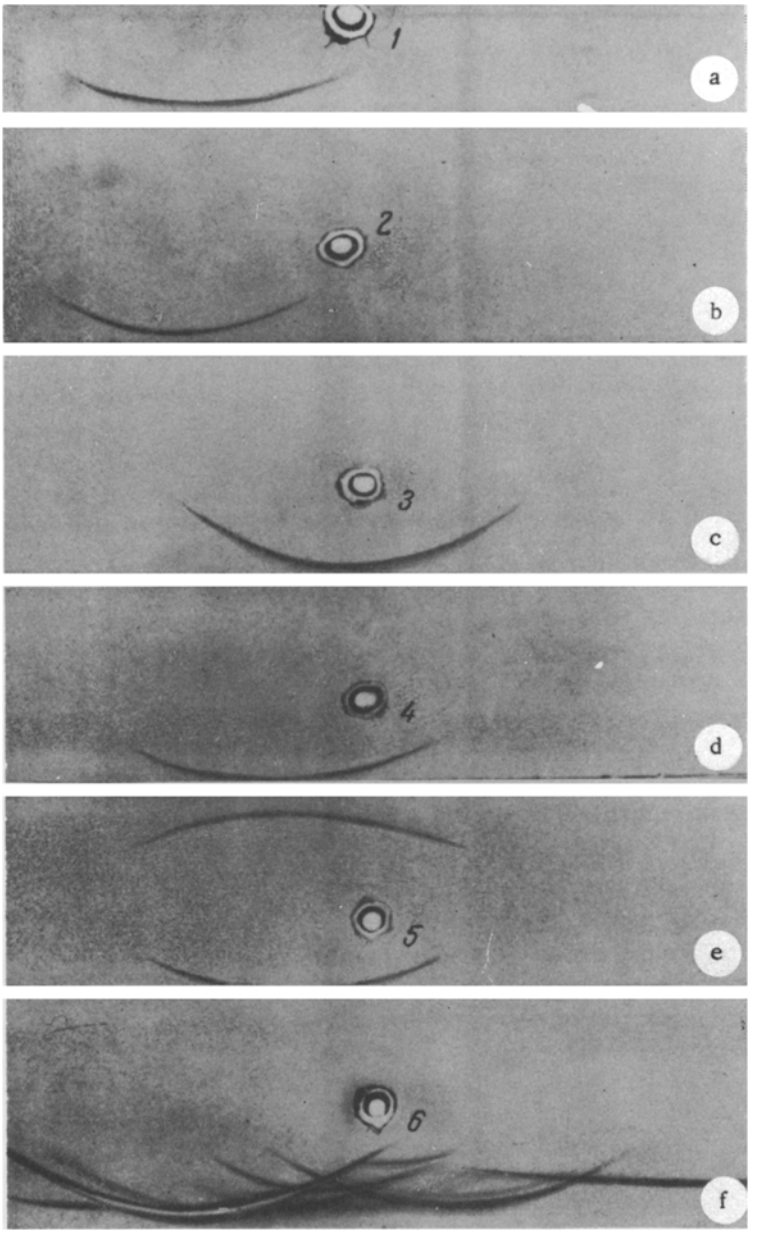

Fig. 1. Comparative immunoelectrophoretic analysis of embryospecific α-globulins. Sera of fetal: 1) pigs, 2) humans, 3) dogs, 4) sheep, 5) cows, 6) adult human. Antisera: a) Anti-fetal pig, b) anti-fetal human, c) anti-fetal dog, d) anti-fetal sheep e) anti-fetal cow, f) antisera against human adult serum.

rats and humans migrated to the α-globulin zone and the embryonic protein of the dog is located in the region of the α_2- and β_1-globulins.

The results of cross serological reactions of embryospecific α-globulins of man and certain animals are presented in the table. Anti-fetal human serum crossreacted with sera of fetal dogs and pigs. However the expression of the reactions (the intensity of the precipitation lines) (Fig. 2, a) was different during the use of differing components. In the same experimental set-up anti-fetal human serum depleted with adult human serum did not react with bovine fetuin which agrees with the data of other authors (6).

Anti-fetal dog sera crossreacted with the production of expressed precipitation lines with embryospecific com-

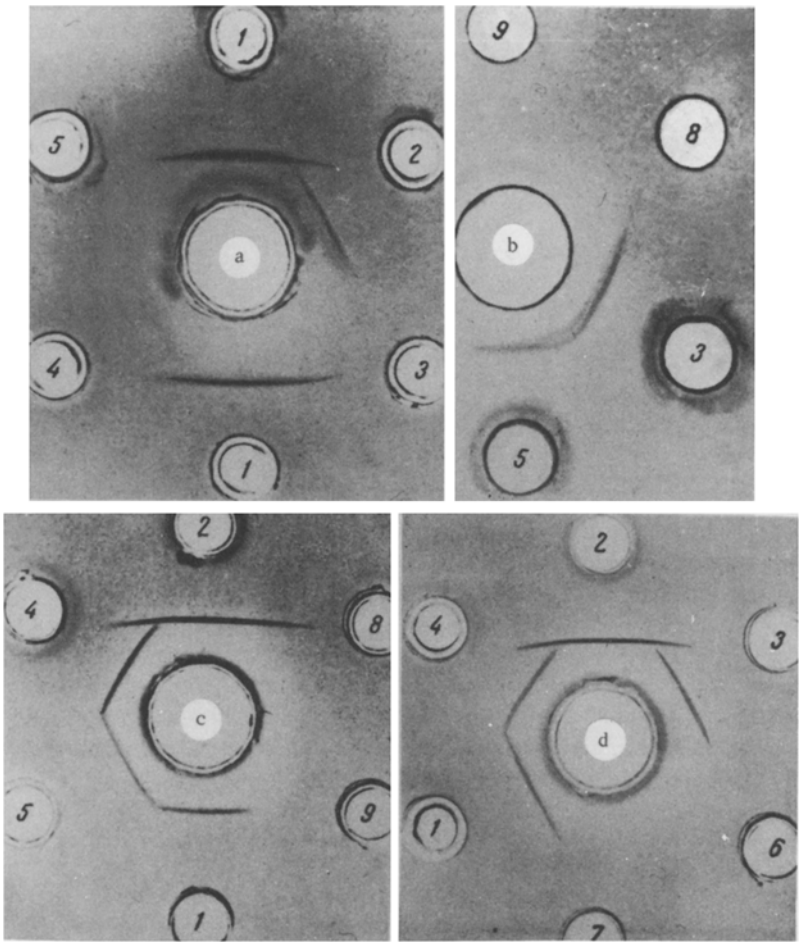

Fig. 2. Precipitation reaction in gel according to Ouchterlony. Antisera: a) anti-human fetal, b) anti-bovine fetal, c) and d) anti-dog fetal. Antigens: 1) fetal human; 2) fetal dog, 3) fetal bovine, 4) fetal pig, 5) fetal sheep. Adult sera (control): 6) dog, 7) human, 8) bovine, 9) sheep.

Results of Precipitation Reactions of Antisera (to embryospecific α-globulins) with Homo- and Heterologous Antigens

Antisera	Antigens							
	fetal human	fetal dog	fetal bovine	fetal pig	fetal sheep	fetal rat	newborn rat	newborn guinea pig
Anti-human fetal	○	+	–	+	–	–	–	–
Anti-dog fetal	+	○	+	+	+	–	–	–
Anti-bovine fetal	–	+	○	+	○	–	–	–
Anti-pig fetal	+	+	+	○	+	–	–	–
Anti-newborn rat	–	–	–	–	–	○	–	–

Notation: ○ reaction completely identical with standard test system; + reaction not completely identical; – visible precipitation lines not observed.

ponents of the sera of humans, cows, sheep and pigs. It is interesting that using these antisera we were able to observe the phenomenon of complete identity of embryonic α-globulins of humans and pigs (Fig. 2, d) and incomplete identity of similar proteins of the fetal serum of pigs and sheep (Fig. 2, c).

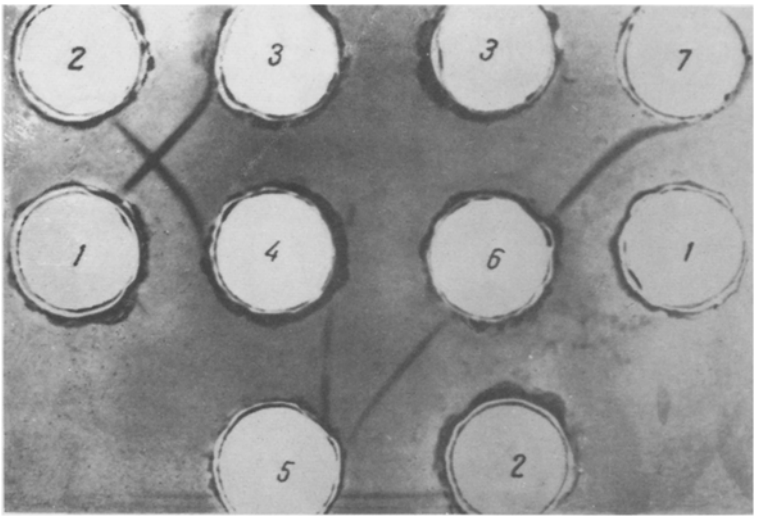

Fig. 3. Precipitation reaction in gel according to "square" (G. I. Abelev modification). Antisera: 1) anti-newborn rat, 2) anti-human. Antigens: 3) fetal rat, 4) fetal human. Normal sera (control): 5) human, 6) rabbit, 7) rat.

During a study of crossreactions using anti-fetal bovine sera it was shown that the embyospecific bovine and sheep α-globulins upon double diffusion in agar formed a fusing arch of precipitation which indicates the complete immunological similarity of the compared proteins (Fig. 2, b). This is, perhaps, a unique case in which serum proteins differing in species relationship displayed complete immunological similarity of antigenic determinants. During the reaction of these same anti-fetal bovine sera with other heterologous embryospecific components the immunological differences showed up very clearly.

A comparison of two complete systems of antigen-antiserum according to G. I. Abelev (1) showed that in embryospecific rat α-globulins similar antigenic determinants characteristic of the embryonic α-globulins of man, dog, cow and pig were absent. During this experiment lines of specific precipitate were formed independent of each other with the formation of a "cross" (Fig. 3).

The results which we obtained showed that similar antigenic determinants are contained in embryospecific α-globulins of man and certain animals (dog, cow, sheep, pig) the amount of which, evidently, varies. By studying the crossreactions by the method of double diffusion in agar it will be possible to establish the species specificity of each of the antigenic groupings in the embryospecific globulins and at the same time to determine more completely the total amount of the antigenic determinants in the native molecule of these proteins.

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