

COMPARATIVE IMMUNOELECTROPHORETIC ANALYSIS
OF THE BLOOD SERUM PROTEINS OF FETAL,
NEONATAL, AND ADULT RATS

A. V. Afanas'eva

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The results of electrophoretic investigations of the serum protein composition of rats in ontogenesis [5] have shown that in the early stages of intrauterine development albumins and β_2 -globulins appear. At this time the α -globulin fraction consists of a single protein, differing in mobility from the α -globulin of the adult animal, and no γ -globulins are found.

Results obtained by the electrophoresis-precipitate method [3] have shown that the appearance of proteins similar to the serum protein of adult rats takes place in fetuses in a different order. The γ -globulins were first found in fetuses weighing 0.32-0.42 g, i.e., in an early stage of development, albumins and α_3 -globulins—shortly before birth, and the γ_1 -globulins in newborn animals. It was shown that, besides the formation of proteins identical with those of adult rats, embryonic proteins appear in the serum at certain stages of development, disappearing after birth.

A. E. Gurvich and N. G. Karsaevskaya [3] included prealbumin and η -globulin among the embryonic proteins, while Zh. G. Shmerling and V. D. Uspenskaya [5] found similar embryonic proteins in the region of the α -globulins.

New data on the formation of the serum proteins in the embryogenesis of rats have been obtained by the method of immunoelectrophoresis in agar. By this method 5-7 antigenic components have been detected in the early stages of development of the rat fetus (0.39-0.8 g) corresponding to nearly all the main protein fractions, except the γ_3 -globulin, of the blood serum of the adult rat [10]. It has also been shown that the α -globulins of fetal serum contain an embryo-specific protein [1, 9].

This paper describes the result of comparative immunochemical investigations of the serum proteins of fetal, neonatal, and adult rats, and also describes the characteristics of the embryo-specific globulin and gives the result of determination of its production at different stages of ontogenesis.

EXPERIMENTAL METHOD

The antigenic composition of the blood serum of fetal, neonatal, and adult rats was compared by the method of immunoelectrophoresis in agar in a type EFA-1 chamber, which was specially adapted for semimicroanalysis by the method of Grabar and Williams [6]. The technique and conditions of immunoelectrophoresis were described earlier by the author [2].

Two types of rabbit antiserum were used for detection of immunity: antiserum No. 92 against the serum of an adult rat, capable of revealing up to 22 antigenic components in adult serum (the precipitation arcs were identified by the scheme proposed for mouse serum by Heremans and co-workers [7]); antiserum No. 38* against the serum of rat fetuses in the second half

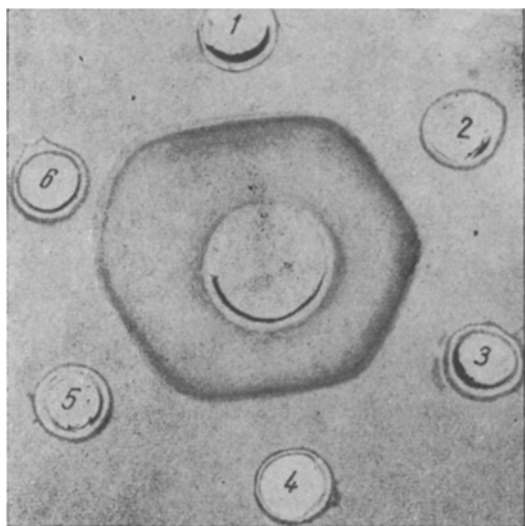


Fig. 1. Titration of ESA-globulin in agar. The central well contains antiserum against ESA-globulin. 1-6) Pooled serum of rat fetuses in different dilutions (from 1:4 to 1:256).

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Immunoelectrophoretic Characteristics of Antigenic Components of Blood Serum of Fetal, Neonatal, and Adult Rats

| Object from which serum was obtained | No. of tests | Weight (in g) | Age (in days) | Prealbumin | Albumin | Globulins | | | | | | |
|--------------------------------------|--------------|---------------|---------------|------------|---------|--------------------------------|------------|------------|-----------|-----------|-----------|----------|
| | | | | | | α_1 | α_2 | α_3 | β_1 | β_2 | β_3 | γ |
| | | | | | | number of antigenic components | | | | | | |
| Adult rats | 12 | — | — | 0—1 | 1 | 1—3 | 3—4 | 2—4 | 2 | 2—4 | 0—2 | 1 |
| Fetuses | 8 | 0,76 | | 1 | (1) | 1 (1) | — | 1 | 1 | 1 | — | (1) |
| » | 6 | 3,4 | | 1 | 1 | 1 | (1) | 2 | 1 | 1 | — | (1) |
| » | 4 | 4,8 | | 1 | 1 | 3 | (1) | 3 | 1 | 2 | 1 | (1) |
| Newborn rats | 5 | 4,4 | 1 | 1 | 1 | 2 | (1) | 2 | 2 | 2 | 1 | (1) |
| » | 8 | 5,5 | 1 | — | 1 | 2 | (2) | 2 | 2 | 2 | — | (1) |
| » | 4 | 5,4 | 1 | 1 | 1 | 2 | (1) | 1 | 2 | 1 | — | (1) |
| » | 2 | 9,4 | 10 | — | 1 | 3 | 2 | 3 | 2 | 2 | — | 1 |
| » | 2 | 13,4 | 15 | — | 1 | 2 | 2 | 1 | 2 | 2 | — | 1 |
| » | 2 | 16,5 | 20 | — | 1 | 2 | 3 | 2 | 2 | 2 | (1) | 1 |

Note: The figures in parentheses give weak antigenic components: serum against adult rats serum was used for their immunological detection.

of pregnancy, and antiserum No. 106 against the serum of newborn rats on the first day of life. After these sera had been exhausted with an excess of serum of adult male rats, they continued to react only with one of the α -globulins of the fetal serum. The monospecific antisera thus obtained against embryonic α -globulin (ESA-globulin) were used for quantitative immunochemical investigations. Pooled serum of rat fetuses was used as antigen, and a Heideberger's precipitation curve was plotted with it. Depending on the size of the precipitate, the content of ECA-globulin was determined in the tested pool serum, knowing that in the equivalent zone the precipitate contains 10% of antigen [8].

The protein concentration in the precipitate was determined by Lowry's method [11], and in the serum refractometrically.

Next, using the same monospecific antiserum, the pooled fetal serum was titrated in agar [4], and the last dilution determined giving a clearly visible precipitation arc, not dissolving during prolonged exposure (Fig. 1).

Knowing the total content of ESA-globulin in the pooled serum, the minimal amount of ESA-globulin forming a visible precipitation arc during titration with 7 different dilutions was determined. In these experimental conditions the last visible precipitation arc was found with an absolute concentration of ECA-globulin of 0.015 mg/ml.

Comparative analysis of the absolute content of ECA-globulin in the sera of fetal, neonatal, and adult rats was carried out in similar experimental conditions. The result was read from the last dilution of the test serum giving a visible precipitation arc.

EXPERIMENTAL RESULTS

The results shown in the table were obtained by comparing the antigenic composition of the blood serum of the fetuses extracted in the early stages of intrauterine development and of newborn rats, with the possible variations in the antigenic composition of the serum of adult animals.

As the table shows, antigenic components corresponding to the main protein fractions of adult rats blood serum could be found in the fetuses in the early period of development. However, their assortment in each main protein fraction was much poorer than in the adult rat. Prealbumins, albumins and β -globulins were seen most clearly in this period, but the α -globulins were indistinct, possible because their concentration was too low. In fetuses at birth and in the newborn animals the antigenic composition of the blood serum became more complex, and by the 10th-20th day of life the process of formation of the serum protein was largely complete.

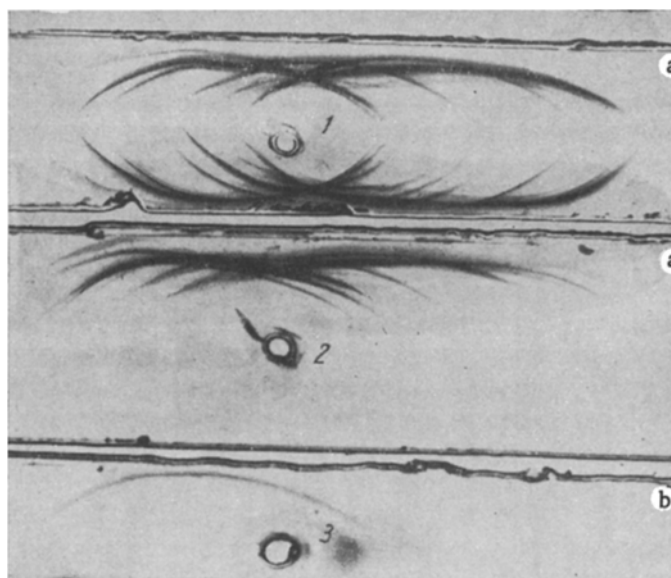


Fig. 2. Immunoelectrophoretic characteristics of ECA-globulin. 1 and 2) Serum of an adult rat; 3) serum of a rat fetus; a—anti-serum against the serum of an adult rat; b—antiserum against ECA-globulin.

Besides the appearance of proteins immunologically similar to the proteins of adult rats, an embryo-specific component was found in the serum of the fetuses, corresponding to γ_1 -globulin in electrophoretic mobility (Fig. 2). The content of ECA-globulin was highest (about 10%) in the fetal serum. After birth the production of ECA-globulin gradually fell, and by the 30th day of life of the newborn rat it had almost completely disappeared.

ECA-globulin was found in very small quantities (0.12 mg/ml) in the serum of a pregnant rat, whereas, none was found in the serum of a nonpregnant rat or of male rats.

The results obtained show that the formation of the protein composition of the blood serum in rat fetuses begins with the simultaneous appearance of proteins immunologically similar to nearly all the main serum fractions of adult rats. Later, the antigenic composition of the individual protein fractions simply becomes more complex. By the 10th-20th day of life of the newborn rat, the formation of the antigenic composition of the blood serum is almost complete.

Besides proteins immunologically identical to the serum proteins of the adult rat, an ECA-globulin appears in fetuses in the early stages of development, but its production falls considerably after birth and ceases altogether by the 35th day of life. Similar results on the dynamics of embryo-specific γ -globulin were obtained by G. I. Abelev and co-workers in the embryogenesis of mice.

ECA-globulin is absent from the serum of nonpregnant rats and of male rats, but is present in small amounts in the serum of pregnant rats. This suggests that ECA-globulin passes from the fetus into the maternal blood through the placental barrier.

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