

IMMUNOCHEMICAL IDENTIFICATION OF A NEW EMBRYO-SPECIFIC  
 $\beta$ -GLOBULIN IN THE BLOOD SERUM OF HUMAN EMBRYOS,  
 FETUSES, AND NEONATES

Yu. S. Tatarinov and V. N. Masyukevich

UDC 612.64'124-088.1

A new globulin has been identified among the serum  $\beta$ -globulins of human embryos, fetuses, and neonates, and described as embryo-specific  $\beta_0$ -globulin. It differs in its antigenic properties and electrophoretic mobility from human embryo-specific  $\beta_1$ - and  $\beta_2$ -globulins.

\* \* \*

It was shown in a previous paper [5] that at least three embryo-specific antigenic components, which are not present in the blood serum of healthy adult persons, can be identified immunochemically among the  $\beta$ -globulins of human fetal serum. Later, among these globulins, an embryo-specific  $\beta$ -glycoprotein [6, 7], with sedimentation constant 10 S [1] and an embryo-specific  $\beta$ -globulin [6, 7] have been identified.

The object of the present investigation was to make an immunochemical study of the third fetal  $\beta$ -globulin, which we have called embryo-specific  $\beta_0$ -globulin (ES  $\beta_0$ -globulin).

#### EXPERIMENTAL METHOD

Antisera against embryo-specific globulins were prepared by repeated reimmunization of rabbits with mixed serum of full-term human fetuses. After the cycle of immunization, between 2 and 4 single reimmunizations were given at monthly intervals [3]. Blood was taken 7-10 days after each reimmunization and the specificity of the antisera obtained was determined.

Two batches of rabbit antisera which, after complete exhaustion with mixed donors' serum, continued to react only with ES  $\beta_0$ -globulin (Fig. 1) were used in the investigation. For the immunoelectrophoretic identification of ES  $\beta_0$ -globulin, mono-specific and polyvalent antisera against certain fetal proteins also were used: embryo-specific  $\alpha_1$ -globulin (ES  $\alpha_1$ -globulin), embryo-specific  $\beta_1$ -glycoprotein (ES  $\beta_1$ -globulin), and embryo-specific  $\beta_2$ -globulin (ES  $\beta_2$ -globulin).

Blood sera from embryos, fetuses, neonates, and infants during the first 3 months of life, and also sera from pregnant and parturient women were titrated by Ouchterlony's method as modified by Khramkova and Abelev [9].

Blood serum was obtained\* from embryos aged 6-10 weeks obtained during medical abortion, from fetuses aged 18-30 weeks after spontaneous abortion, and from full-term neonates and also from infants during the first 3 months after birth. Blood sera from pregnant women before abortion and in the prenatal period, as well as individual blood sera from donors of both sexes, were used as control.

\* Blood was taken by N. V. Mesnyankina, L. F. Parfenova, and N. N. Silishcheva, to whom the authors are grateful.

Department of Biochemistry, A. V. Lunacharskii Astrakhan Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 68, No. 10, pp. 52-55, October, 1969. Original article submitted January 13, 1969.

TABLE 1. Immunochemical Analysis of ES  $\beta_0$ -Globulin in Blood Serum of Embryos, Fetuses, Neonates, and Pregnant Women

Blood sera	Period of pregnancy (in weeks)	No. of samples of sera	Immunochemical test for ES $\beta_0$ -globulin	
			positive	negative
Embryos	6-8	10	7	3
Embryos	9-12	10	8	2
Fetuses	18-30	12	12	0
Neonates	40	115	114	1
Pregnant women	40	115	3	112
Donors: women				
men	control	30	0	30
	control	30	0	30

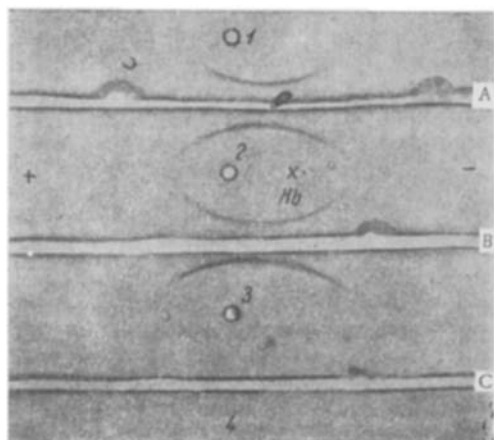


Fig. 1.

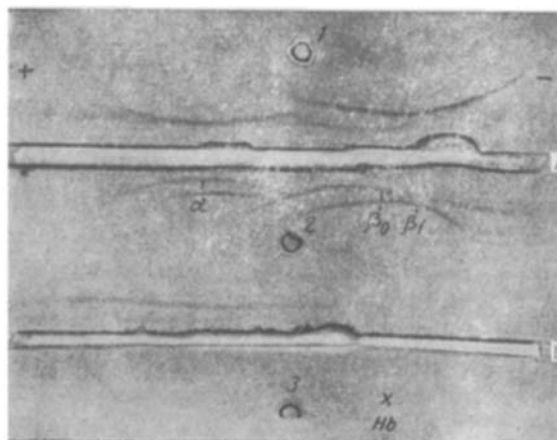


Fig. 2.

Fig. 1. Immunoelectrophoretic characteristics of ES  $\beta$ -globulin (1% Difco agar, Gendon's veronal-medinal buffer, voltage 140 V, current 50 mA, duration 30 min). 1, 2, 3, 4) Blood sera from fetuses of different ages; A, B) rabbit antifetal sera exhausted with donor's serum; C) donor's blood serum; xHB) zone of migration of hemoglobin.

Fig. 2. Comparative immunoelectrophoretic analysis of embryo-specific  $\alpha$ - and  $\beta$ -globulins. 1, 2, 3) Blood sera of fetuses of different ages; A) rabbit antifetal serum exhausted by donor's serum; B) donor's serum.  $\alpha_1$ ,  $\beta_0$ , and  $\beta_1$ ) Embryo-specific globulins.

## EXPERIMENTAL RESULTS

During immunoelectrophoresis on agar, ES  $\beta_0$ -globulin is detected in the cathode part before hemoglobin (Fig. 2), while the ES  $\beta_1$ -globulin described previously [1, 6, 7] possesses the electrophoretic mobility of hemoglobin.

Production of ES  $\beta_0$ -globulin evidently begins in the early stages of embryo genesis. Whatever the case, this component begins to be found in human embryonic serum at the 6th-8th week of development (Table 1), but ES  $\beta_0$ -globulin, in contrast to ES  $\beta_1$ -globulin and ES  $\beta_2$ -globulin, is found in nearly all fetal and neonatal sera, i.e., the synthesis of this serum protein takes place throughout the period of intra-uterine development. In the frequency of its detection, ES  $\beta_0$ -globulin resembles the previously described embryo-specific  $\alpha_1$ -globulin [6, 8, 10, 11]. After birth, production of ES  $\beta_0$ -globulin decreases, and it may perhaps cease altogether during the 3rd month of postnatal life (Table 2). Detection of ES  $\beta_0$ -globulin in human fetal serum and its absence in normal adult human serum indicate that it is a typical embryonic serum protein, the function of which, like that of other embryo-specific globulins described previously, has received little study.

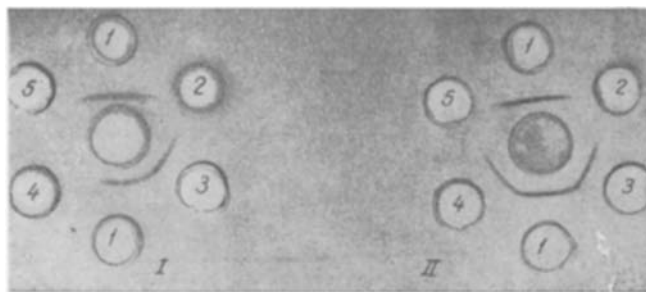


Fig. 3. Titration of embryo-specific  $\beta$ -globulin with standard test system. 1) Antigen of test system (serum of 20-week fetus diluted 1:20 with physiological saline); 2) donor's sera; 3) neonatal sera; 4, 5) sera of pregnant women. Exhausted antisera in center (I-II).

TABLE 2. Immunochemical Analysis of ES  $\beta_0$ -Globulin in Blood Serum of Infants during First 3 Months after Normal Birth

Time after birth (in months)	No. of samples of sera	Immunochemical tests for ES $\beta_0$ -globulin	
		positive	negative
to 1	16	14	2
1-2	15	12	3
2-3	5	2	3

The few cases in which ES  $\beta_0$ -globulin was found in the blood serum of pregnant women are interesting (Fig. 3). In 3 of the 115 blood sera of pregnant women investigated, ES  $\beta_0$ -globulin was found (Table 1). The origin of the ES  $\beta_0$ -globulin in the serum of pregnant women requires special investigation. It may be supposed that, in certain cases, ES  $\beta_0$ -globulin penetrates from the fetal circulation into the maternal blood stream, or that conditions are created in the body of the pregnant woman favoring resumption or a marked increase of the production of this protein. This latter possibil-

ity may be due to the regenerative activity of the liver parenchyma, as has been shown [2, 4] in cases of the discovery of ES  $\beta_1$ -globulin in the active phase of cirrhosis of the liver. The preliminary results now obtained also support this hypothesis. For instance, during the investigation of 25 patients with cirrhosis of the liver, ES  $\beta_0$ -globulin was found in 4 cases.

The immunochemical identification of ES  $\beta_0$ -globulin provides a means for the comparative analysis of the clinical value of immunochemical tests for embryo-specific  $\beta$ -globulins in diseases of the liver and also in other diseases.

#### LITERATURE CITED

1. S. S. Vasileiskii, *Biokhimiya*, No. 5, 959 (1966).
2. A. M. Nogaller, Yu. S. Tatarinov, and F. E. Vishnevetskii, *Vopr. Med. Khimii*, No. 1, 20 (1968).
3. M. Raiskii, *Khar'kovsk. Med. Zh.*, 20, No. 8, 142 (1915).
4. Yu. S. Tatarinov, *Vopr. Med. Khimii*, No. 2, 218 (1964).
5. Yu. S. Tatarinov, *Vopr. Med. Khimii*, No. 6, 584 (1964).
6. Yu. S. Tatarinov, *Vopr. Med. Khimii*, No. 3, 98 (1965).
7. Yu. S. Tatarinov, *Vopr. Med. Khimii*, No. 1, 37 (1967).
8. Yu. S. Tatarinov, V. N. Masyukevich, N. V. Mesnyakina, et al., *Akush. i Gin.*, No. 8, 20 (1967).
9. N. I. Khramkova and G. I. Abelev, *Byull. Éksperim. Biol. i Med.*, No. 12, 107 (1961).
10. C. G. Bergstrand and B. Csar, *Scand. J. Clin. Lab. Invest.*, 8, 174 (1956).
11. G. de Muralto and D. L. Roulet, *Helv. Paediat. Acta*, 16, 517 (1961).