IMMUNOCHEMICAL IDENTIFICATION OF A NEW β_1-GLOBULIN IN THE BLOOD SERUM OF PREGNANT WOMEN

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A new β_1-globulin has been identified in the blood serum of pregnant women. It is absent from the serum of men and nonpregnant women, but is a constant protein component of the blood serum of women throughout pregnancy.

Immunochemical methods of investigation have revealed additional antigenic components in the blood serum during pregnancy which are absent from the serum of men and nonpregnant women. Among these components, placental α_2-globulin [2, 4, 11, 12] and embryo-specific globulins [7, 8] have been identified. During immunoelectrophoretic analysis of the blood sera of pregnant women some workers [1, 3, 5, 6, 10, 14] also have observed an additional precipitation line in the zone of α-globulins without, however, specifying its origin.

In the investigation described below a new β_1-globulin, identified by the writers by means of an antiserum against blood serum proteins of women with normal pregnancy, was studied.

EXPERIMENTAL METHOD

Antisera were obtained by immunization of rabbits with mixed blood serum from pregnant women. The first injection (0.3 ml serum) with Freund's adjuvant was given subcutaneously. With each subsequent injection the dose of antigen was increased by 0.2 ml. The whole cycle of immunization took 3 weeks. Every week three injections of antigen were given, the place of injection being alternated (subcutaneously, intramuscularly, intraperitoneally). Blood was taken 7-10 days after the last injection and an antiserum obtained. The antiserum was exhausted with mixed donor's serum (1 ml antiserum was treated with from 0.3 to 0.5 ml donor's serum). The mixture was incubated at 37°C for 2 h, then left in a refrigerator for 15-20 h. After centrifugation the supernatant was used as monospecific antiserum for immunoelectrophoretic and immunodiffusion analysis. Only those antisera which, after exhaustion, continued to react with individual fractions of the blood serum proteins of pregnant women were used in the experiment.

Immunoelectrophoresis was carried out in a chamber for high-voltage electrophoresis in agar gel. As a rule, during immunoelectrophoresis, the blood sera of pregnant women, when developed with antiserum, formed two precipitation arcs of different degrees of intensity: one in the zone of α_2-globulins and another in the zone of β_1-globulins.

Immunodiffusion analysis was carried out by Ouchterlony's method [13] as modified by Khramkova and Abelev [9]. The fraction of β_1-globulins isolated from mixed serum of parturient women by preparative electrophoresis in agar gel, was used as antigen for the standard test system. For mass analysis, mixed blood serum of pregnant women, first diluted with physiological saline to the optimal concentration of the sought antigen, was used as antigen of the standard test system. Usually dilutions of 1:12-1:16 were chosen.

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TABLE 1. Results of Titration of β_1-Globulin in Blood Serum of Pregnant Women, Fetuses, and Newborn Infants

Blood serum	No. of samples	Result of determination of β_1-globulin		Percent of positive results
		positive	negative	
Serum of women (period of pregnancy):				
6-8 weeks	77	75	2	97.4
9-12 weeks	84	84	0	100
13-30 weeks	12	12	0	100
39-40 weeks	56	56	0	100
Sera of fetuses and newborn infants:				
14-30 weeks	25	2	23	8
39-40 weeks	270	23	247	8.5
Serum of donors:				
women	165	0	165	0
men	92	0	92	0

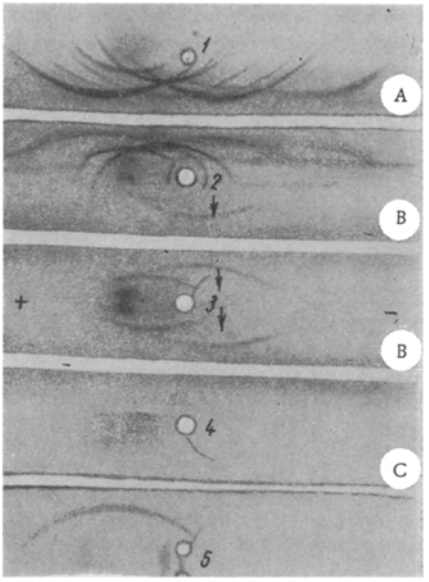

Fig. 1. Comparative electrophoretic characteristics of the new β_1-globulin in blood serum of pregnant women. Individual blood sera: 1, 2, 3) of parturient women; 4) of a nonpregnant woman; 5) of a fetus. Antisera: against blood serum of parturient women before (A) and after (B) exhaustion with donors' serum; C) against embryo-specific α_1-globulin. Arrow points to precipitation line of β_1-globulin.

EXPERIMENTAL RESULTS

Antisera against blood serum proteins of parturient women differed in their specificity and in their content of precipitating antibodies. During immunoelectrophoresis, at least two antigenic components not detectable under the same experimental conditions in control blood sera were discovered in the blood serum of the parturient women. One of these components possesses the electrophoretic mobility of α-globulins and was previously identified by the writers [9] as serum α_2-globulin. A second antigenic component with the electrophoretic mobility of adult hemoglobin was discovered in the zone of β-globulins. This component, described as β_1-globulin, was found in all blood sera of parturient women and could be detected by ordinary immunoelectrophoretic analysis. During immunodiffusion analysis with standard test system or with β_1-globulin, it was found that the analogous antigenic component is also found in nearly all sera of pregnant women (Fig. 2), starting from the 6th week of pregnancy (Table 1), although in a relatively lower concentration.

During titration of sera of pregnant women, β_1-globulin was detected at the 6th-8th week of pregnancy in a titer of 1:8, at the 18th-20th week in a titer of 1:32, and at the 39th-40th week in a titer of 1:256. These results indicate an increase in the production of β_1-globulin as the stage of pregnancy advances.

It is evidently rare for the β_1-globulin to pass from the maternal blood through the placental barrier into the fetal blood stream. As Table 1 shows, the reaction for β_1-globulin was positive in two of 25 cases and 23 of 270 cases respectively in blood sera of fetuses and newborn infants. The reaction was recorded by deviation of the precipitation line of a standard test system (Fig. 2), indicating an extremely low level of β_1-globulin in the fetal blood sera. Titration of control sera (healthy adult men and nonpregnant women) using a sensitive standard test system gave negative results. It can be concluded that β_1-globulin is either completely absent in control sera or present in a concentration undetectable by the methods of immunodiffusion analysis used, i.e., less than 0.5 mg%.

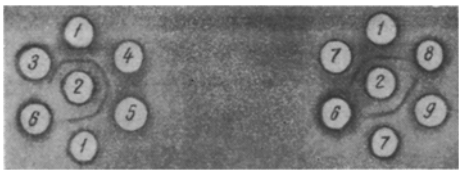

Fig. 2. Titration of new β_1-globulin with a standard antigen – antibody test system. 1) Standard antigen: solution of β_1-globulin isolated from blood serum of parturient women; 2) antiserum against β_1-globulin. Blood sera: 3) neonatal; 4) of women at 6th week of pregnancy; 5) of women at 8th week of pregnancy; 6) of man; 7, 9) of parturient women (dilution 1:160);8) of nonpregnant women.

The results show that the β_1-globulin now identified immunochemically is a constant component of the blood serum of pregnant women, and its determination can be used as a serologic test for the early diagnosis of pregnancy.

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