BIOSYNTHESIS AND PRODUCTION OF SPECIFIC β_1-GLOBULIN IN RATS DURING PREGNANCY

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Specific β_1-globulin of pregnancy was found in the blood serum of rats on the 6th-7th day of pregnancy. Its concentration reached a maximum toward the end of pregnancy. It was no longer detected on the 3rd-4th postnatal day. The site of synthesis of this globulin is the placenta. Other organs of pregnant rats were unable to incorporate radioactive amino acids into this protein in vitro.

KEY WORDS: β_1-globulin of pregnancy; tissue culture; autoradiography; placenta.

It was shown previously [2, 9, 15] that among the serum proteins of the "pregnancy zone" in rats there is a specific β_1-globulin, which is found during pregnancy but is not present in the blood serum of nonpregnant animals. A similar β_1-glycoprotein has been identified in the blood serum of pregnant women [3, 5] and in extracts of the placenta [6, 12]. Comparative immunodiffusion analysis has shown that the specific β_1-globulin (SBG) of pregnancy possess marked immunologic similarity in rats and mice [9], and also in monkeys and man [15]. Results indicating that human SBG is synthesized in the placenta and is localized in cells of the cyto- and syncytiotrophoblast have been obtained [7, 8, 14].

The object of this investigation was to determine the volume of production and the site of synthesis of SBG during pregnancy in rats.

EXPERIMENTAL METHOD

Antisera against rat β_1-globulin were obtained by the method described previously [9]. Immunodiffusion analysis was carried out by Ouchterlony's method [16] in the modification of Khramkova and Abelev [10]. Grabar and Williams' method of immune-electrophoresis [13] was used.

Samples of placental tissue (7-14 and 15-19 days of pregnancy) were obtained from rats under ether anesthesia. Pieces of placenta (1-1.5 g) were taken for culture, washed, shredded with scissors and, as recommended by Paul [4], cultured at 37°C with 3 ml of Eagle's culture medium containing a mixture of labeled ^{14}C-amino acids from *Clorella* digest; the total activity of the sample was 10 μCi. Incubation continued for 18-24 h. Some organs of pregnant rats (liver, kidneys, heart, spleen) were cultured in the same way. The supernatant was cooled and lyophilized, and pieces of tissue were homogenized in Tris-glycine buffer, pH 8.6, with Triton X-100 and Tween-80, frozen and thawed three times, and the resulting supernatant was lyophilized. Control samples, treated in the same way, were kept for 18-24 h at −4°C, and the supernatant was then lyophilized. The experimental and control freeze-dried preparations were dissolved in 0.5 ml physiological saline and used for immunoautoradiographic study. Tissue culture was carried out in the same way in medium No. 199 with the addition of fetal calf serm and antibiotics. From 12 samples were taken 28 ml of medium No. 199, 5 ml fetal calf serum, and 3.5 ml *Chlorella* digest, with additional solubilization of the ^{14}C-labeled lysine of amino acids. The total activity of the medium was 1150 μCi/36.5 ml. From 20 to 40 μCi was added to the culture medium.

Radioimmunoelectrophoresis and radioimmunodiffusion were carried out [1, 11] both with and without the addition of blood serum from pregnant rats, because the test system showed up well with culture fluid also. Immunodevelopment continued for 2 days in a moist chamber

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TABLE 1. Results of Immunochemical Determination of Specific β_1-Globulin in the Blood Sera of Pregnant, Parturient, and Nonpregnant Female and Male Rats

Material	No. of animals	No. of positive results		Titer
		absolute	%	
Blood serum of rats after mating:				
on 2nd-3rd day	38	0	0	
on 4th-5th day	40	0	0	
on 6th-7th day	40	5	12,5	1:1
Blood serum of pregnant rats (second half of pregnancy)	53	53	100	1:16—1:128
Blood serum of rats after parturition:				
on 1st-2nd day	8	8	100	1:1—1:8
on 3rd-4th day	43	0	0	
Blood serum of males and nonpregnant females	80	0	0	

TABLE 2. Results of Determination of Synthesis of Specific Rat β_1-Globulin in Culture Fluid after Incubation of Different Tissues with Addition of Labeled ^{14}C-Amino Acids

Time of pregnancy, days	Tissue cultured				
	placenta	liver	kidney	heart	spleen
7—14	6/3	1/0	1/0	1/0	1/0
15—19	6/5	1/0	1/0	1/0	1/0
Total	12/8	2/0	2/0	2/0	2/0

Legend. Number of individual tests in numerator; number of positive results in denominator.

and was followed by washing in buffered physiological saline, with three or four changes, after which the agar was dried under chromatography paper under a fan. High-sensitivity RF-3 film was applied to the dreid slides and pressed between two slides of corresponding size. Autoradiographic development continued for 20-30 days.

EXPERIMENTAL RESULTS

As Table 1 shows, specific β_1-globulin of pregnancy was first found in the rats' sera on the 6th-7th day; its concentration reached a maximum toward the end of pregnancy, and on the 3rd-4th postnatal day it had already disappeared from the mother's blood serum.

The placentas for culture were taken from the animals on the 7th-14th and 15-19th days of pregnancy, for it is at this time that the concentration of this protein in the blood serum is highest. The results of immunoautographic determination of SBG by means of labeled amino acids are given in Table 2. They show that radioactive antigenic components immunochemically similar to β_1-globulin could be found in the tissue culture of the placenta starting from the 7th-14th day of pregnancy. Incorporation of labeled amino acids was shown to take place only in the tissues of the placenta, whereas in other organs of these same pregnant rats (liver, kidney, heart, spleen) no radioactive components similar to the specific rat protein of pregnancy could be found (Fig. 1). It was shown by the double diffusion method with a standard test system (Fig. 2) that the radioactive β_1-globulin was immunochemically identical whether with or without the addition of native blood serum of pregnant animals. To rule out the possibility of nonspecific adsorption of labeled amino acids on the SBG, in control experiments tissue of placenta and other organs was incubated at —4°C, and in this case

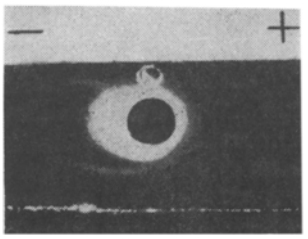

Fig. 1

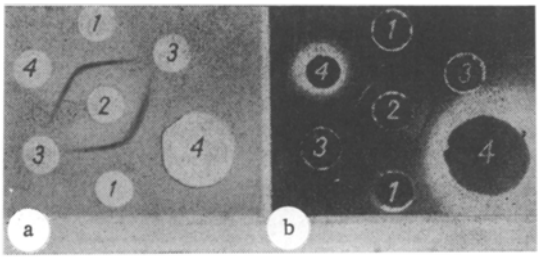

Fig. 2

Fig. 1. Immunoautoradiographic analysis of rat SBG contained in culture fluid of placenta. Conditions of electrophoresis: 1.5% Difco agar, veronal-medinal buffer, pH 8.6; ionic strength 0.05; 130 V, 50 mA, duration 40 min. Immunodevelopment of antispecific β_1-globulin for 20 h, exposure for autoradiography 30 days.

Fig. 2. Radioimmunodiffusion analysis of rat SBG contained in culture fluid of placenta. a: 1, 2) Test system against specific β_1-globulin; 3) physiological saline; 4) culture fluid from rat placenta. b: The same figure as a, exposure for autoradiography 20 days.

no radioactive components immunochemically similar to specific β_1-globulin were found. Similar results were obtained with human trophoblastic β-globulin, when it was shown that it is synthesized in the placenta [8] and localized, as shown by the immunoflorescence method, in cyto- and syncytiotrophoblastic cells [7, 8].

It can be concluded from these results that specific β_1-globulin begins to be found in the blood serum of pregnant animals on the 6th-7th day of pregnancy, its concentration reaches a maximum by the end of pregnancy, and on the 3rd-4th postnatal day it has already disappeared from the maternal blood stream. The site of its biosynthesis is the placenta. Other organs of pregnant rats (liver, kidney, heart, spleen) do not synthesize this protein.

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