PRODUCTION OF EMBRYOSPECIFIC α-GLOBULIN IN DOGS DURING INTRAUTERINE LIFE AND EARLY POSTNATAL LIFE

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Investigations previously conducted by the author [2] have led to the discovery of two proteins among the embryonic serum globulins of the developing fetus; these proteins differ from the serum proteins of the adult dog in their antigenic properties. These new proteins are distributed in globulin zones α_3 and α_4 during immunoelectrophoretic separation and have been identified as α-globulins. This present work deals with the effect of the age of the fetus on the amount of one of these embryospecific globulins which is produced and also with the possibility of its passing through the placenta into the maternal bloodstream.

EXPERIMENTAL

I. Production of monospecific antiserum: Antisera obtained by the immunization of rabbits with sera from 7-8 weekold dog fetuses were used in these experiments. Provisionally denoted as embryospecific α-globulin ESA. After exhaustion of excess serum by the plasma from adult male dogs, the antiserum was shown to contain only one ESA-globulin (Fig. 1). II. Determination of ESA-globulin content: The results of our experiments with plasma from 7-8 week old fetuses and the monospecific antiserum derived therefrom enabled us to construct a Heidelberger precipitation curve. The amount of precipitate is determined by the ESA-globulin content of the whole plasma from 7-8 week old fetuses because, according to a well-known proposition [4], the precipitate contains 10% antigen in the equivalent zone. The concentration of protein in the precipitate was determined by Lowry's method [5] and in the

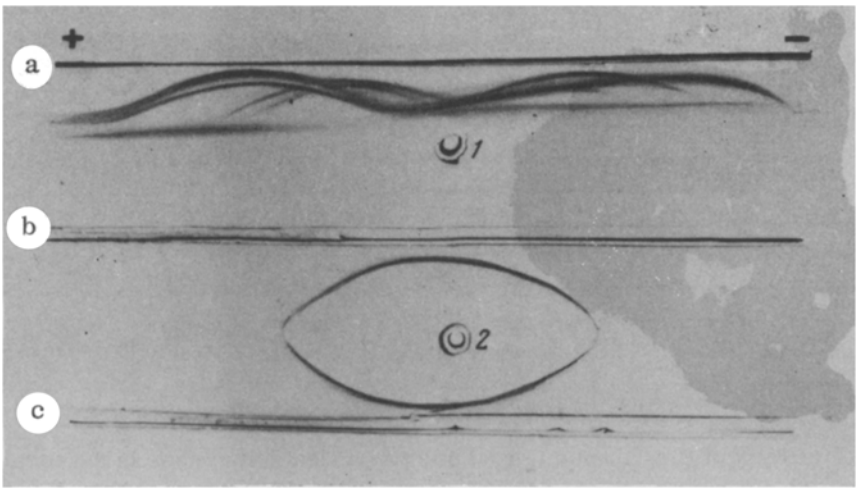

Fig. 1. Immunoelectrophoretic characteristics of ESA-globulin. 1) Plasma of adult dog; 2) plasma of dog fetus; a) antiserum against plasma of adult dog; b) against ESA-globulin.

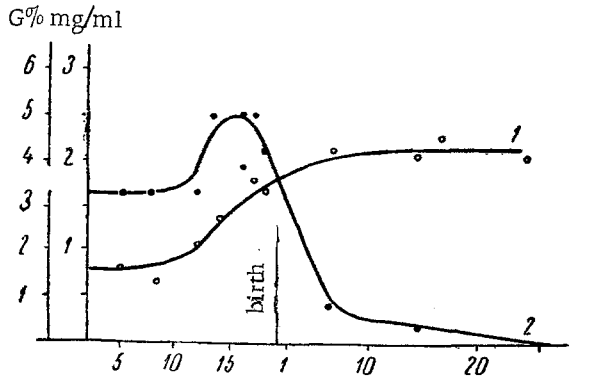

Fig. 2. Proportion of ESA-globulin (in mg/ml) and total protein (in g%) in blood plasma from dog fetuses and puppies at various stages of development. 1) Total protein; 2) ESA-globulin.

whole blood plasma by refractometry. III. Titrimetry of ESA-globulin in agar: The ESA-globulin content of embryo-plasma and puppy plasma at various stages of development were determined by titrimetry in agar using a standard test-system [1, 3]. Plasma from 7-8 week old fetuses at a dilution of 1:4 was used as an antigen in the test-system; undiluted monospecific serum against ESA-globulin served as the antiserum.

The test-system was selected in accordance with the optimal relationship between antigen and antibody; under such conditions they produced a sharp zone of precipitation that did not dissolve after prolonged exposure. The chosen test-system proved to be capable of developing when the experiment was set up in "sevens" using ESA-globulin at a serum dilution of 1:12,000 (i.e., with an absolute concentration of ESA-globulin of 0.00021 mg/ml).

A comparative analysis of the absolute ESA-globulin content of the plasma of embryos and puppies of all ages has yielded a similar pattern of results. The results took into consideration the last dilution of the serum which was capable of bringing about a deviation in the angle of the line of precipitation of the test serum.

In order to evaluate the permeability of the placental barrier with respect to ESA-globulin, we undertook a quantitative estimation of the amount of this protein in blood plasma derived from the arteries and veins of the uterus and also from veins of the umbilicus prior to parturition of the puppy.

RESULTS

As is seen from Fig. 2, the intensity of ESA-globulin production by the embryo increases up to the time of parturition. The ESA-globulin content of the blood plasma increases simultaneously with the increase in total plasma protein, such that the ESA-globulin content remains at 8-10% of the total plasma proteins throughout the whole period of intrauterine development of the embryo.

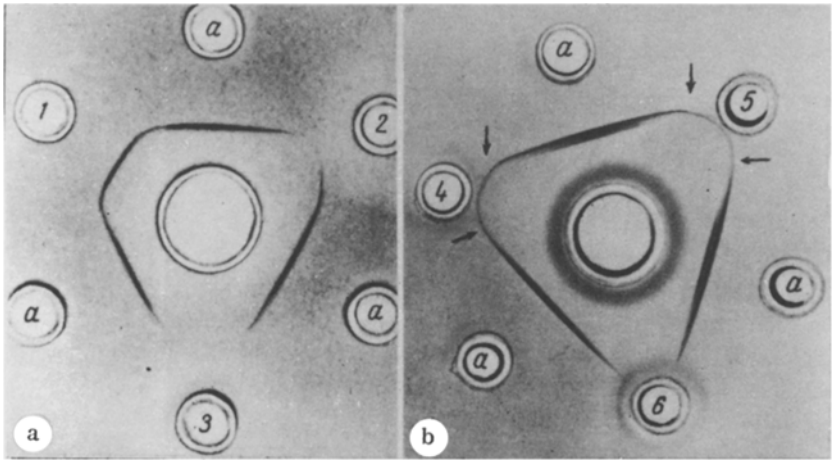

Fig. 3. Titrimetry of ESA-globulin in agar using a standard test-system. In the central holes—antiserum against ESA-globulin; a) antigen of standard test-sytem (plasma from 7-8 week old fetus, diluted 1:4). Blood plasma of pregnant dog, taken from uterine vein (1), from left ventricle (2), from inferior vena cava (4), from inferior vena cava (5); 3,6) plasma from blood of male dog. Arrows indicate change in the line of precipitation in standard test-system.

During the first few days after parturition the synthesis of ESA-globulin noticably falls, so that by the 23rd day of life the puppy's blood plasma contains only a negligible amount of this protein and from the 30th day onwards ESA-globulin cannot be detected. At the same time, the amount of total plasma protein continues to increase, although the content in an 18-20 day old puppy average (4.5% g) is less than that in an adult dog (average 6.89%).

The titrimetric results for ESA-globulin in various tests carried out on pregnant dogs are represented in Fig. 3. As can be seen from this figure, the highest amount of this protein is contained in blood plasma derived from the uterine veins. It was only possible to detect minimal amounts of ESA-globulin in blood from the inferior vena cava by means of titrimetry employing standard test-systems, and when plasma from the male dogs was tested we were unable to find any ESA-globulin at all.

The results we have obtained indicate that the most intensive synthesis of ESA-globulin takes place during the second half of intrauterine development, that after birth the production of this protein is sharply reduced, and that in the 30-day old puppy production has ceased completely. In addition we have established the possibility of ESA-globulin passing the placental barrier into the maternal blood stream.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
