

IMMUNOELECTROPHORETIC ANALYSIS OF SERUM PROTEINS
IN THE ONTOGENESIS OF THE DOG

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A previous investigation [1] showed that embryonic serum albumin of the dog possesses immunological properties identical with those of the analogous fraction of the serum proteins of the adult animal. By means of the method of immunoelectrophoresis in agar it is possible to detect as many as 20 antigenic components [3] in the dog's blood serum, but the times of their appearance during the period of intrauterine development of the animal have not yet been studied. In the present paper we describe the results of comparative immunoelectrophoretic investigations of the serum proteins of dog embryos and fetuses at different periods of development, and of adult animals.

METHOD

Immunoelectrophoresis was carried out in agar by Grabar's method [2] in the following conditions: 1% agar prepared in buffer by Laurell's method [4], voltage 200 V, current 10 mA, total immersion of chamber, duration 5-6 h. To obtain antisera, one group of rabbits was immunized with the blood serum of adult dogs. The antiserum used in the investigations was capable of detecting 22 antigenic components in the serum of an adult dog. Another group of rabbits was immunized with the serum of 7-8-week dog fetuses. The sera thus obtained against embryonic proteins were exhausted with an excess of serum of an adult dog. The monospecificity of the exhausted antiserum was verified immunoelectrophoretically. A comparatively small volume of test serum (5-8 μ l) was used for immunoelectrophoresis; 0.2 ml of antiserum was placed in the groove. The preparations were incubated in a humid chamber at 37° for 36 h, irrigated with 0.9% sodium chloride solution for 3 days, and photographed in scattered light. The identification of the precipitation arcs was carried out in accordance with the scheme described by Korinek and Paluska [3].

RESULTS

It is clear from Fig. 1 that the blood serum of the fetuses during the early periods of intrauterine life contains a limited range of antigenic components. The results given in the table demonstrate that the antigenic composition of the blood serum gradually becomes more complex in the course of growth and maturation of the fetus. In the first place individual antigenic components are found in the zones of albumin, and β_1 and α -globulins. At birth, the antigenic composition of the fetal blood serum is more complex, but as before no β_2 -globulins are present in the serum, and γ -globulins are only beginning to appear.

After birth the formation of serum protein fractions and the increase in the complexity of their antigenic composition continued in the puppies. It should be noted that in all the puppies on the first days after birth the γ -globulin gave a clear precipitation arc, whereas the arc formed by the β_2 -globulins was ill defined in most puppies. The possible explanation of this result is the very low concentration of this protein in the blood serum. Otherwise the antigenic range of the serum proteins in the month-old puppies corresponded almost completely to the antigenic composition of the serum protein of the adult dog.

Immunoelectrophoretic Characteristics of Antigenic Components of Blood Serum of Fetuses, Puppies and Adult Dogs

Source of blood serum	Number of tests	Length of fetus (in cm)	Age (in weeks)	Albumins	Globulins						
					α_1	α_2	α_3	α_4	β_1	β_2	γ
					Number of antigenic components						
Dog	15			1-3	1-3	2-3	3-4	1-2	2-4	1-2	1
Fetuses	6	7-13	4-6	1	0-2	1*-2	0-2	0-1	2	0	0
"	18	15-18	7-8	1-3	1-2	1-3	1-4	1-2	1-3	0	0-1*
Puppies	2		1	1	1	1	4	2	3	1*	1
"	1		2	1	1	2	3	1	1	0	1
"	2		3 $\frac{1}{2}$	2	1*	3	4	2	3	0	1
"	3		4 $\frac{1}{2}$	2	2	2	4	2	2	1*	1

*The antigenic components were ill defined; antiserum to the serum of an adult dog was used for immunization.

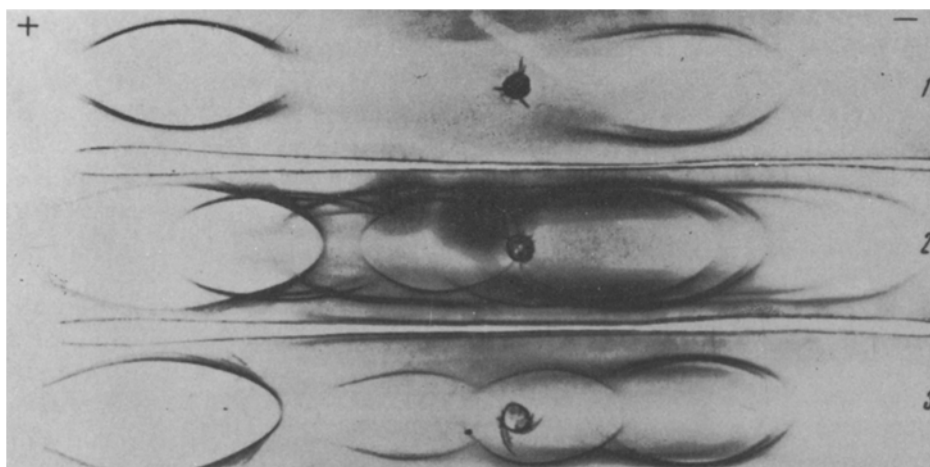


Fig. 1. Immunoelectrophoresis of blood serum of fetuses, puppies, and an adult dog, treated with antiserum to serum proteins of an adult dog. 1) serum of fetus (4-5 weeks of development), 2) of adult dog, 3) of puppy (3 $\frac{1}{2}$ weeks of development).

To obtain more accurate information regarding the similarity between the individual antigenic components of the blood serum of the fetuses and adult dogs, the method of specific absorption was used. Antiserum to the serum of an adult dog was absorbed by an excess of the serum of fetuses of different periods of development. In this way it was shown that the number of antigenic components in the fetal serum immunologically similar to the serum proteins of adult dogs increased with growth of the fetus (Fig. 2).

In the same way the method of exhaustion of the sera was used to detect specific embryonic proteins. For this purpose a monospecific serum was used, containing antibodies only against the embryonic proteins of fetuses of 7-8 weeks of development. This antiserum detected 2 embryonic proteins in the zones of the α_3 - α_4 -globulins (Fig. 3). These embryonic globulins appeared in the serum of the fetuses after the 4th week of their development and disappeared on the 15th-20th day after birth, while no analogous proteins were found in the serum of the adult dogs.

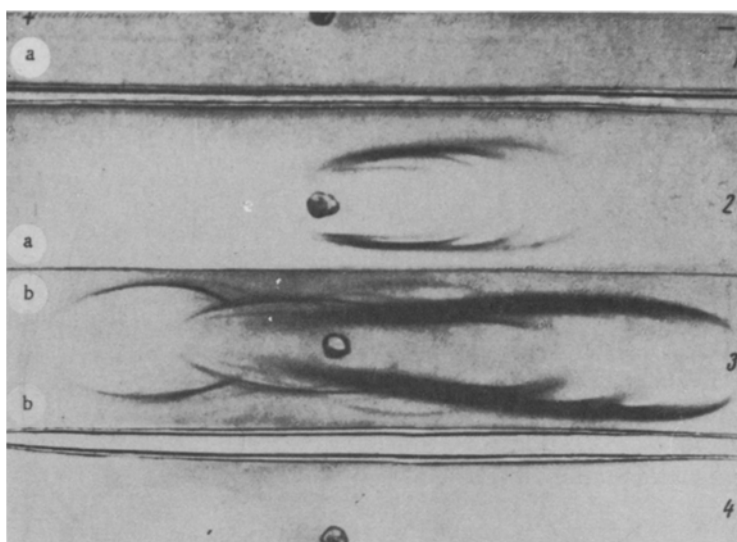


Fig. 2. Immunoelectrophoresis of blood serum of adult dogs and fetuses of different ages. 1) serum of a fetus of 7-8 weeks of development, 2,3) of a dog, 4) of a fetus of 4-5 weeks of development; a) antiserum to serum of an adult dog, exhausted by excess of serum of a fetus of 7-8 weeks of development, b) exhausted with serum of a fetus of 4-5 weeks of development.

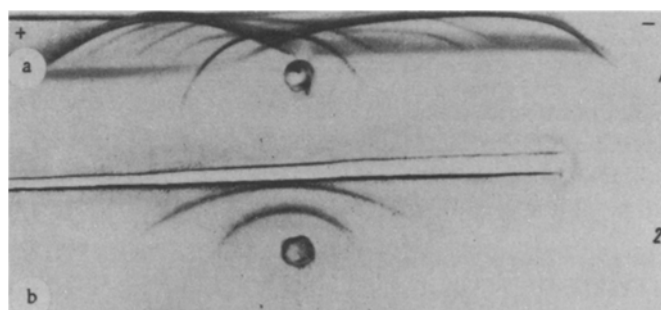


Fig. 3. Immunoelectrophoretic characteristics of embryonic proteins detected by antiserum against serum of 7-8 week fetuses. 1) serum of a dog, 2) of a fetus of 4-5 weeks of development; a) antiserum to serum of an adult dog, b) antiserum to serum of fetuses of 7-8 weeks of development, exhausted by excess of serum of an adult dog.

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