# IMMUNOCHEMICAL CHARACTERISTICS OF CANNINE SERUM ALBUMIN IN ONTOGENESIS

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The development of immunochemical methods for research in embryology has opened up new prospects for the solution of problems relating to the synthesis of individual protein fractions during ontogenesis. Undoubted interest attaches to the application of comparative immunochemical study of the serum proteins of fetal and adult animals in this connection.

Sporadic papers are to be found in the literature, dealing with the comparative immunochemical study of serum proteins of certain animals during ontogenesis [6, 16]. These papers deal, however, only with the qualitative immunochemical evaluation of individual protein fractions, without giving a quantitative assessment of their degree of immunochemical similarity.

We undertook a systematic comparative examination of the antigenic properties of the serum albumin of fetuses, puppies, and adult dogs, applying methods of qualitative and quantitative immunochemical indentification of proteins.

## EXPERIMENTAL METHODS

Antisera were prepared by immunization of rabbits against dog serum or serum albumin. For this purpose rabbits were given 3 intravenous injections weekly of progressively increasing amounts (6-16 mg) of potassium alum-adsorbed antigen, for 3-4 weeks. Blood was taken on the 6th or 7th day after the last injection, and was tested for precipitin activity. If the precipitin reaction of the serum was not strong enough, the rabbits were reimmunized 1 month later, by a single intravenous injection of 20-25 mg of antigen (large doses of antigen were administered by the Besredka procedure). When the precipitin content was adequate (2-4 mg/ml), the antisera were used for the experiments.

Fractionation of the sera and isolation of albumins were performed by starch slab electrophoresis (1 x 13 x 26 cm), using veronal buffer, pH 8.6, with a potential difference of 450 V, for 48 h. After completion of electrophoresis, prints were made of the slab on chromatography paper, which was stained with bromphenol blue. Sections of the slab corresponding to the albumin bands on the print were cut out, and the albumins were extracted with the same buffer as had been used for electrophoresis.

The purity of the albumins so obtained was checked by means of paper electrophoresis.

Comparative immunochemical analysis of the electrophoretically homogeneous albumins of fetuses, puppies, and adult dogs was performed by the immunoelectrophoresis method. For this purpose we used an EFA-1 equipment, specially adapted for macro- and micro-analysis according to Grabar and to Scheidegger [9, 15]. The conditions used for immunoelectrophoresis were: 1% agar, prepared with a special buffer [11], 200 V, current strength 10 ma for the whole cell; duration 5-6 h.

The albumins of fetuses and puppies were also compared with those of adult dogs by a minor modification of Ouchterlony's method of double diffusion in agar [14].

The precipitation arcs were photographed without preliminary staining, in scattered light [8].

For a quantitative assessment of the degree of immunological similarity of the albumins we set up a series of precipitation reactions, using as the antigen increasing concentrations of the albumins under examination. For each

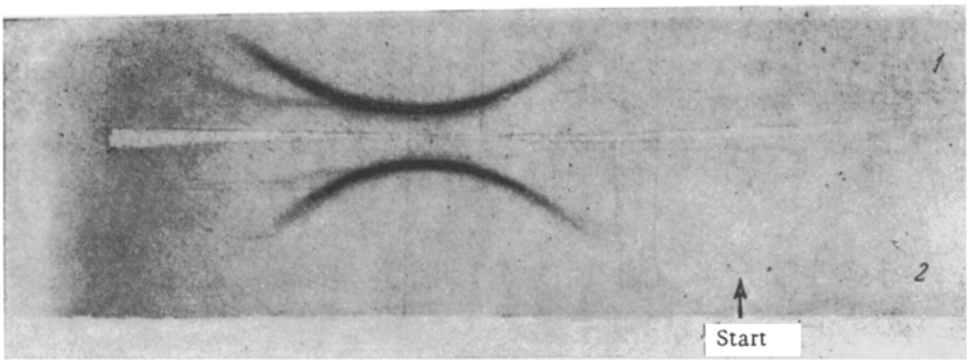


Fig. 1. Immunoelectrophoregram of the serum albumin of a 6-week fetus (1) and of an adult dog (2), reacting with antiserum to adult dog serum albumin.

specimen we constructed a curve expressing dependence of the amount of precipitate on the amount of antigen added, according to Heidelberger [10].

The degree of immunological similarity was derived from readings on the ascending branch of the precipitation curve, and in the optimum zone, comparing the curves obtained for fetuses and puppies with those of precipitation of albumins of adult dogs.

The protein contents of the solutions and precipitates were determined by the Lowry method [12], as described by A. E. Gurvich and R. B. Kapner [5].

The fractional composition of the sera was determined by a paper electrophoresis method, in an equipment of the type described by Flynn and de Mayo, as modified by A. E. Gurvich [4]. The total protein content of sera was determined by a refractometric method.

## EXPERIMENTAL RESULTS

As is evident from the table, the albumin content of fetal serum is about one-third that for adult dogs. It rises as gestation progresses, but even in 35-day-old puppies the albumin content is still below that of adult dogs.

Immunoelectrophoretic analysis, using anti-albumin serum and antiserum to total dog serum proteins, showed that the albumins tested gave a clear-cut precipitation arc, which is evidence of their homogeneity and of their identical mobility in the electric field (Fig. 1). When examined by double diffusion in agar, according to Ouchterlony, all the fetal albumins, taken at different stages of embryonic development, gave single precipitation bands, which then coalesced to give precipitation arcs (Fig. 2). Taking into consideration published data on methods of immunochemical identification of various proteins [2, 7, 13, 14], it may be concluded that the serum albumins of fetuses, puppies, and dogs constitute a common group of antigens. The degree of immunological similarity of these albumins may be judged

Serum Albumin Content of Dogs, Puppies, and Fetuses, and the Degree of Immunological Similarity

| Animal material | Age (in weeks) | Number of animals | Albumins | |
|---|---|---|---|---|
| | | | mean content in serum, g% | amount of protein precipitated by antiserum to adult dog serum albumin, % |
| Dogs* | Adult | 12 | 2.91 ($\sigma \pm 0.24$) | 100.0 |
| Fetuses | 5-6 | 26 | 1.17 ($\sigma \pm 0.29$) | 95.1 |
| Fetuses | 7-8 | 36 | 1.26 ($\sigma \pm 0.23$) | 95.5 |
| Puppies | 0-2 | 7 | 1.89 ($\sigma \pm 0.55$) | 95.3 |
| Puppies | 2-5 | 9 | 2.04 ($\sigma \pm 0.48$) | 96.2 |

*Blood samples were taken before removal of fetuses, or in the postnatal period

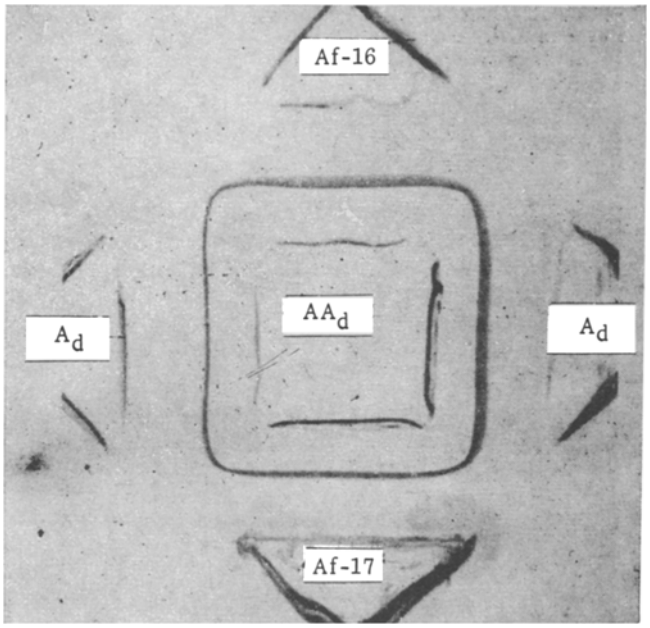


Fig. 2. Double diffusion in agar, according to Ouchterlony. Af-16) Albumin from a 6-week fetus; Af-17) albumin from a 5-week fetus; $A_d$) albumin from an adult dog; $AA_d$) antiserum against albumin from an adult dog.

from the results of quantitative immunochemical analysis. We found that the serum albumin of fetuses and puppies gave about the same amount of precipitate with antiserum to adult dog albumin, at the same part of the ascending branch of the curve, and at the optimum zone of antigen concentration, as did the albumin of adult dogs. The immunological differences between the different albmins under comparison varied, on the average, by from 3.8 to 4.9%, which lies within the limits of experimental error of the method. Thus a quantitative examination of the antigenic similarities of the albumins has confirmed our conclusion that the serum albumin of dogs is immunologically the same at all stages of ontogenesis. The placenta of dogs is of the endothelial-chorionic type, and is impermeable to maternal blood proteins [1, 3]. It may be supposed, on the grounds of their immunological similarity, and of the impermeability of the placenta to maternal blood proteins, that synthesis of albumins takes place in the fetus in such a way that the fetal protein is immunologically identical with that of the adult animal.

## SUMMARY

Qualitative and quantitative comparative immunochemical studies have been made of the serum albumins of fetuses, puppies, and adult dogs, using Ouchterlony's diffusion method, and immunoelectrophoresis according to Grabar; Heidelberger's curve was used in evaluation of quantitative results. The albumins were isolated by starch slab electrophoresis.

No immunological differences were found between the albumins. It is suggested that the antigenic determinant of serum albumin synthesized by the fetus is identical with that functioning during adult life.

## LITERATURE CITED

1. I. A. Arshavskii. Physiology of the Circulation during Intrauterine Life [in Russian], M., 1960.
2. V. S. Gostev. Chemistry of Specific Immunity [in Russian], M., 1959.
3. Ya. G. Gubarevich. Veterinary Obstetrics and Gynecology [in Russian], M.–L., 1960.
4. A. E. Gurvich. Labor. delo, 1955, No. 3, p. 3.
5. A. E. Gurvich and R. B. Kapner. Labor. delo, 1958, No. 2, p. 23.
6. A. E. Gurvich and N. G. Karsaevskaya. Biokhimiya, 1956, No. 6, p. 746.
7. V. A. Parnes. Byull. éksper. biol., 1957, No. 11, p. 117.
8. Yu. A. Khavkin. Labor. delo, 1961, No. 2, p. 45.
9. P. Grabar. Biokhimiya, 1957, No. 1-2, p. 49.
10. M. Heidelberger. Bact. Rev., 1939, Vol. 3, p. 49.
11. C. B. Laurell, S. Laurell, and N. Skoog, Clin. Chem., 1956, Vol. 2, p. 99.
12. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. biol. Chem., 1951, Vol. 193, p. 265.
13. J. Oudin, C. R. Acad. Sci. (Paris), 1946, Vol. 222, p. 115.
14. O. Ouchterlony, Acta path. microbiol. scand., 1948, Vol. 25, p. 186.
15. J. Scheidegger, Int. Arch. Allergy, 1955, Vol. 7, p. 103.
16. H. Vainer and V. Andresco, Arch. roum. Path. exp., 1960, Vol. 19, p. 29.