IMMUNOELECTROPHORETIC ANALYSIS OF WATER-SOLUBLE ANTIGENS OF THE CHICKEN RETINA

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The chicken retina contains six serum and nine tissue antigens, of which two are organ-specific and seven interorgan antigens, differing in their spectrum of distribution in other organs and tissues of chickens.

The system of tissues of the vertebrate eye is frequently used in experimental embryology as an object for the study of the principles of organogenesis. Immunological methods, making it possible to assess the degree of differentiation of tissues at the level of specific antigens, have been used during recent years in such investigations. However, analysis of the antigenic properties of the retina, occupying a central position in the system of regulation of growth and development of the eye tissues, has been undertaken only sporadically [3, 4].

The object of the present investigation was to study water-soluble antigens of the definitive chicken retina.

EXPERIMENTAL METHOD

The chicken retina was homogenized in 5 volumes of distilled water, and the extract obtained after centrifugation was lyophilized and kept in a closed vessel at 4°. The yield after lyophilization was 6% of the fresh weight of the tissue. Before the experiments, the lyophilized product was dissolved in 0.1 M tris buffer (pH 8.6) to a concentration of 40 mg/ml. Extracts were obtained in the same way from the liver, spleen, brain, heart, and muscles of chickens.

A total extract of the retina was used to immunize 4 rabbits which were injected every week for 3-6 months with 1-2 ml of extract mixed with Freund's complete adjuvant. Another group of rabbits was immunized with electrophoretic fractions of the retina. The retinal extract was separated into 6 fractions by electrophoresis in agar gel. Pieces of agar with the corresponding fractions were minced, mixed with

TABLE 1. Relative Electrophoretic Mobility (m_r) of Tissue Antigens of the Chicken Retina

Retinal antigen	No. of observations	Electrophoretic mobility (M±m)	Retinal antigen	No. of observations	Electrophoretic mobility (M±m)
Ia	23	155,0±1,5	3	4	120,0±1,4
Ib			4	34	86,7±0,8
Anode	23	155,0±1,5	5	18	43,9±0,6
Middle	6	115,7±0,4	6	45	35,6±0,2
Cathode	5	79,5±0,4	7	9	25,4±0,8
2	29	113,1±1,0	8	37	16,1±0,4

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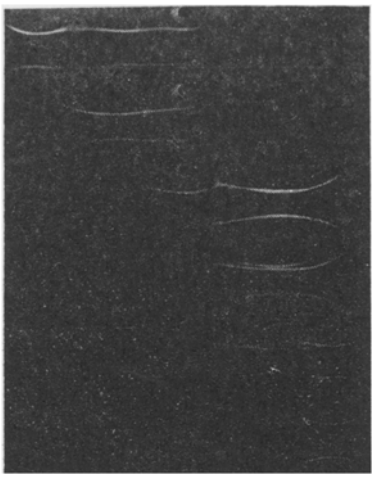

Fig. 1. Immunoelectrophoresis of tissue antigens of the chicken retina. Wells contain retinal extract; gutters contain antisera against 6 different electrophoretic fractions of the retina.

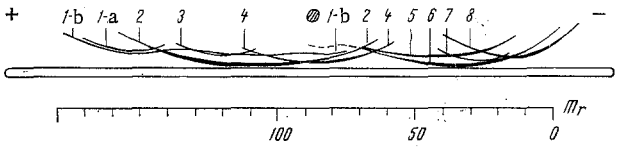

Fig. 2. Composite scheme of distribution of tissue antigens of the chicken retina in immunoelectrophoresis.

an equal volume of Freund's adjuvant, and injected subcutaneously twice into 6 rabbits, with an interval of three weeks between injections. The antisera had titers of 1:100-1:1000.

Immunoelectrophoresis was carried out in agar gel made up in tris buffer (pH 8.3-8.6; μ 0.1) with a voltage gradient of 4.6 V/cm, for a duration of 90 min. The electrophoretic mobility of the antigens was calculated in conventional units (m_r) relative to the mobility of human serum albumin [6]. In an alternative method of immunoelectrophoresis [5], sera against the retina were exhausted with extracts from chicken organs and blood serum.

EXPERIMENTAL RESULTS

In the reactions with chicken retina, antisera obtained against the total extract of the retina formed from 8 to 10 precipitation marks. However, after exhaustion with chicken blood serum, only 2-4 arcs of weak intensity remained in the reactions. Antisera against total extract from the chicken retina thus contained antibodies mainly against antigens of serum origin and a few antibodies against retinal tissue antigens.

A more varied spectrum of tissue antigens was obtained in the chicken retina by means of antisera against electrophoretic fractions of the retina. Each of the 6 antisera formed not more than 3 precipitation arcs in the reaction with total extract of the retina (Fig. 1). Altogether, by means of these antisera, 9 antigens differing in electrophoretic mobility and in the shape of the precipitation bands which they formed, were detected in the chicken retina by means of these antisera (Fig. 2). The values of the mean electrophoretic mobility of the individual antigens given in Table 1 show that, besides antigens with low mobility (antigen 8, $m_r = 16.1$), the retina also contains antigens whose rate of migration during electrophoresis is extremely high (antigen 1a, $m_r = 155$).

Retinal antigens with low and average values of electrophoretic mobility formed regular precipitation arcs during immunoelectrophoresis. Conversely, antigens with high mobility (antigens 1b, 2) formed complex, extended bands, indicating that the retina contains populations of molecules with similar antigenic specificity but heterogeneous as regards charge. For instance, the precipitation band corresponding to antigen 1b consisted of 3 successive arcs merging at their ends and formed by 3 electrophoretic fractions of the retina with mobilities of 155, 115, and 80 conventional units, respectivity.

To determine the organ specificity of the retinal tissue antigens, the antisera for immunoelectrophoresis were absorbed by extracts from chicken organs and blood serum. In this way, the presence of a particular retinal antigen in other tissues was judged from disappearance of the corresponding precipitation arc from the antigenic spectrum of the retina. The results of this series of experiments (Fig. 3) show that antigens 1a and 1b-8 of the chicken retina are tissue antigens and have no similarity with serum antigens. Evidently only two of these antigens (2 and 4) can be regarded as organ-specific, because the corresponding precipitation arcs were still present in reactions with the retina after exhaustion of the antisera by all tissue extracts used in these experiments. All the remaining tissue antigens detected in the chicken retina possessed interorgan specificity. However, it is an interesting fact that these antigens differed in the width of their distribution among chicken organs and tissues. For instance, antigens with relatively low electrophoretic mobility (antigens 5, 6, 7, and 8) were found in all the tissues investigated. Conversely, antigens 1a and 1b were found only in the retina and in the brain tissues of chickens and not in other organs. An intermediate position as regards the width of organ specificity was occupied by antigen 3. This is present in the retina, in brain and muscle tissue, and in the spleen but is absent from the chicken liver.

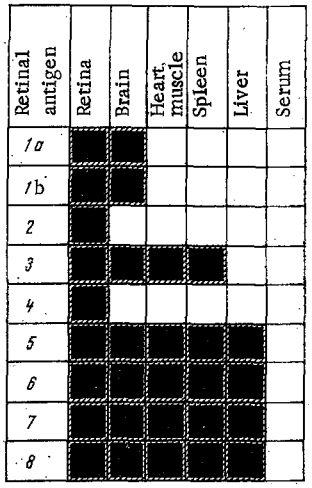

Fig. 3. Organ specificity of tissue antigens of the chicken retina. Results of 144 tests with antisera from 6 rabbits are given in this table. A black square denotes the presence, a white square the absence of the antigen.

It can be concluded from these results that the chicken retina possesses a complex assortment of water-soluble antigens and contains serum, organ-specific, and interorgan antigens.

The presence of serum antigens is evidently explained by the anatomical proximity of the retina to the vascular membrane of the eye.

The discovery of organ-specific antigens in the chicken retina is in agreement with the views of Hess and Römer [3] regarding antigenic organ specificity of the retina in mammals, which they expressed over 60 years ago on the basis of analysis of the cytotoxic properties of antiretinal sera. The two antigens discovered in the chicken retina, with electrophoretic mobilities of $m_r = 113.1$ and $m_r = 86.7$, respectively, are evidently among the carriers of the organ-specific properties of this tissue.

The data described above, together with the results of investigation of antigens of other organs [1, 2, 4], demonstrate the heterogeneity of interorgan antigens, resulting from their unequal distribution in different tissues. The presence of antigens in certain tissues, such as antigens 1a and 1b in the chicken retina and brain, for example, perhaps reflects some degree of functional or structural similarity between these tissues. However, special investigations are necessary before this problem can be solved.

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