ORGAN SPECIFICITY OF RETINAL TISSUE ANTIGENS IN FOWLS

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Ten tissue antigens were found in the retina of fowls: one organ-specific and 9 interorganic. The interorganic retinal antigens were unequally distributed in the other tissues and organs, for 3 of them were characteristic of the tissues of the eye and brain and the other 6 were found in many other organs and tissues of the fowls studied, i.e., they were common organ antigens. From the point of view of antigenic similarity, the brain and iris are closest to the retina, but retinal antisera revealed only common organ antigens in the lens.

The tissue system of the vertebrate eye represents a convenient model for studying the character of interaction between tissues in the course of organogenesis. Among these interactions, those between the developing lens and retina have attracted the special attention of investigators [5]. The study of the role of the retina in morphogenesis of the lens received a fresh impetus with the introduction of immunochemical methods [7]. However, by comparison with the lens, the antigenic properties of the retina have been inadequately studied.

EXPERIMENTAL METHOD

Experiments were carried out on lyophilized extracts from the retina, lens, iris, brain, liver, spleen, kidney, muscle tissue, and lung of fowls, which were kept at 4°C. To prepare the initial solutions, the lyophilized extracts were dissolved in 0.1 M tris buffer, pH 8.6, to a concentration of 40 mg/ml. Antisera against water-soluble retinal antigens of the fowls were obtained by immunizing rabbits with 6 fractions of retinal extract isolated by preparative electrophoresis in agar gel [2]. Antisera absorbed with normal fowl blood serum were used in the tests. Exhaustion with lyophilized extract of fowl serum was carried out in test tubes and also by Björklund's method in agar gel, with a control of completeness of removal of antibodies against the serum antigens in each case.

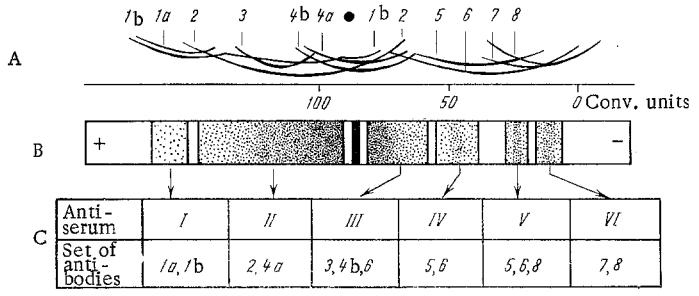

Fig. 1. Composite antigenic spectrum of fowl retina revealed by antisera against different electrophoretic fractions of the retina. Immunoelectrophoretic spectrum of retinal antigens (A), electrophoresis of retinal extract (B), and assessment of antibodies in antisera against 6 retinal fractions (C).

The tests used included Ouchterlony's double diffusion test in gel, titration in agar gel with serial dilutions of extracts within a concentration interval of lyophilized substance from 40 to 0.03 mg/ml, and immunoelectrophoresis on plates measuring 9 ×12 cm in 0.1 M tris buffer, pH 8.2-8.6, in a voltage gradient of 4.6 V/cm [4].

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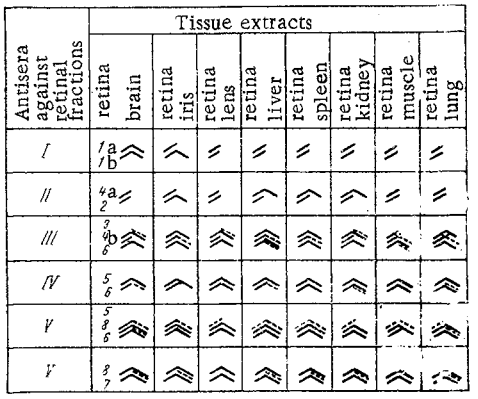

Fig. 2

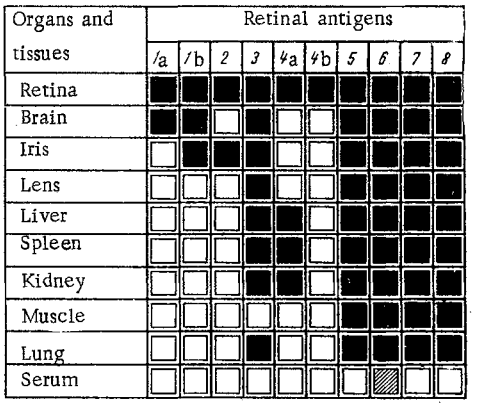

Fig. 3

Fig. 2. Results of crossed tests between antisera against retinal fractions and extracts from fowl organs and tissues. Combined results of 4 series of tests with each antiserum are given; broken line denotes weak and additional precipitation bands in heterologous systems.

Fig. 3. Organ specificity of tissue antigens of fowl retina. Black square denotes antigen present; white square antigen absent; shaded square denotes partial identity of retinal antigen with serum antigens.

EXPERIMENTAL RESULTS

Each of the antisera against the 6 electrophoretic retinal fractions contains a small set of antibodies and formed 2 or 3 precipitation bands on immunoelectrophoresis and in the Ouchterlony test. To characterize the sets of antibodies in the antisera, they were compared in Ouchterlony tests with optimal dilutions of retinal extracts for detecting each precipitation band. The results of these experiments showed that antisera against retinal fractions I and II did not contain effects of antibodies identical with the other antisera. Conversely, besides individual antibodies against the homologous fraction, antisera III-VI contained antibodies against several tissue antigens of neighboring fractions. On the whole, with the aid of these antisera 10 tissue antigens were found in the fowl retina. On immunoelectrophoresis, the retinal antigenic spectrum lay in the zone of electrophoretic mobility from 155 to 16 conventional units (Fig. 1). The antigen with the highest mobility was described as antigen 1, and the rest were numbered in order of decreasing electrophoretic mobility. Two antigens detected by antisera II and III possessed identical electrophoretic mobility. However, the Ouchterlony tests showed that they are not immunologically identical. On this basis they were described as antigens 4a and 4b.

To study the organ specificity of the retinal tissue antigens, extracts from 8 different organs and tissues of fowls were titrated with each antiserum, and the identity of the antigens detected in the other tissues was studied in crossed Ouchterlony tests with retinal antigens (Fig. 2). The results show (Fig. 2) that antisera I and II reacted only with the tissues of the eye and brain, whereas antisera containing antibodies against antigens of the cathodic fractions of the retina formed precipitation bands with all tissue extracts tested. In some cases antisera III, V, and VI, when tested with extracts from the iris, brain, liver, and spleen, formed more precipitation bands than in the homologous reaction. These additional antigens formed very weak precipitation bands in the Ouchterlony tests, making their comparison with retinal tissue antigens difficult. Since antisera were obtained against the retina, it can be postulated that the fowl retina contains, besides the 10 antigens already identified, other antigens present in smaller concentrations or possessing weak antigenicity.

The retinal tissue antigens differed in the width of their spectrum in other tissues and organs of the fowls (Fig. 3). Of all these antigens, only 4b could be regarded as organ-specific, for it was found in the retina and was absent in the other tissues. Interorganic antigens 1a, 1b, and 2, which were present only in the tissues of the eye and brain, were closely similar to it in specificity. Antigen 1a, for instance, was found in the retina and in the brain, antigen 1b in the retina, brain, and iris. According to the results of immunoelectrophoretic analysis [2], antigen 2 was regarded as organ-specific for it was not found in the series of parenchymatous organs.

However, when the distribution of this antigen in the eye tissues was investigated by Ouchterlony's method, the results showed that it is also present in the fowl iris (Fig. 3).

Retinal antigens 5, 6, 7, and 8, which were found in all extracts studied, constitute another group of interorganic antigens. On this basis they were classed as interorganic antigens of broad specificity or, to use the terminology of other workers, common organ antigens [1, 3, 8] or heteroorganic [6] antigens. Antigens 3 and 4a were also close to the antigens of this group in their degree of interorganic specificity. Antigen 3 was found in all organs except muscle tissue. Antigen 4a was detected in extracts of the retina, kidney, liver, and spleen. It is an interesting fact that antiserum III against this antigen formed the densest precipitate in tests with extracts of the retina and kidney. In the other two organs this antigen was found only in trace concentrations.

It can thus be concluded from the results of this investigation of the organ specificity of retinal antigens that besides organ-specific and interorganic antigens of narrow specificity, the fowl retina also contains interorganic antigens with a wide spectrum of distribution in other tissues.

The results shown in Fig. 3 can also be used to compare the antigenic similarity between the tested tissues. The largest number of retinal antigens was found in the iris and brain, probably in connection with the presence of both structural elements in these tissues, on account of their common origin in embryogenesis. The antigenic similarity between the retina and brain is also confirmed by the results of investigation of the antigenicity of individual electrophoretic fractions of extracts from these organs. Immunization experiments with extract of fowl retina showed that the anodic fractions possess lower antigenicity with respect to rabbits than the cathodic fractions. This rule has also been found to apply to brain tissue [9].

The antigenic similarity between lens and retina is limited to the group of interorganic antigens of wide specificity; it does not contain retinal antigens of narrow specificity, by contrast with the iris and brain. From the standpoint of the lens, the retina is thus just as foreign as the parenchymatous organs. If functional and morphogenetic links between the lens and retina in the system of the eye are taken into consideration, the study of the antigenic differentiation of the lens and retina from the comparative aspect could be of particular interest.

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