ANALYSIS OF WATER-SOLUBLE ANTIGENS OF THE HEN IRIS

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Three serum and 12 tissue antigens were discovered in the hen iris by immunodiffusion and immunoelectrophoresis. Among the tissue antigens five were characteristic of the eye tissues only: a specific antigen of the iris, an antigen found in the iris and retina, and three antigens characteristic of the lens (α, β, Δ crystallins). The remaining seven were inter-organ antigens, differing in their distribution in the tissues and organs investigated; they included one which is specific for muscle tissue.

KEY WORDS: hen iris; water-soluble antigens.

The vertebrate eye is a convenient object with which to study tissue interaction during organogenesis. With the aid of immunochemical markers, the changes taking place during development of the tissue anlagen can be reliably determined. Of the various eye tissues, the lens and retina have been studied immunochemically in detail [1, 3, 7]. However, information on the iris is given only in isolated communications [6, 9]. Its antigenic characteristics, however, could be interesting because of the possible role of the cells of the iris in structural changes in the eye tissues.

Water-soluble antigens of the hen iris were investigated.

EXPERIMENTAL METHOD

Lyophilized extracts of the iris, retina, lens, brain, liver, and muscle tissue of hens were tested. To prepare working solutions the freeze-dried preparations were dissolved in 0.1 M tris buffer, pH 8.3, usually to a concentration of 40 mg protein/ml.

Antisera were obtained by immunizing rabbits with the separate fractions of the iris, which were isolated by preparative electrophoresis by a method similar to that described in [3, 4]. The extract of the hen iris was separated into five fractions by electrophoresis in agar gel (Fig. 1A). The corresponding zones of agar were cut out, homogenized, mixed with an equal volume of Freund's adjuvant, and injected subcutaneously into five rabbits at intervals of 4 weeks. On average the material for one immunization was obtained from fractionation of 40 mg of the freeze-dried tissue. Antisera obtained after the second reimmunization of rabbits were used in this investigation.

Besides the above-mentioned antisera, antisera against two anodal fractions of the hen retina [2] and an antiserum against hen blood serum obtained from the Research Institute of Forensic Medicine, Ministry of Health of the USSR, were used.

Immunoelectrophoresis in agar gel [4] was carried out on slabs measuring 9 × 12 cm in tris buffer, pH 8.3, with a voltage gradient of 4.6 V/cm. Ouchterlony's microprecipitation method and the titration in agar gel test [10] were used for immunodiffusion analysis. Absorption was by Bjorklund's method [5], and the agar around the wells and gutters was saturated with extracts from hen organs and serum.

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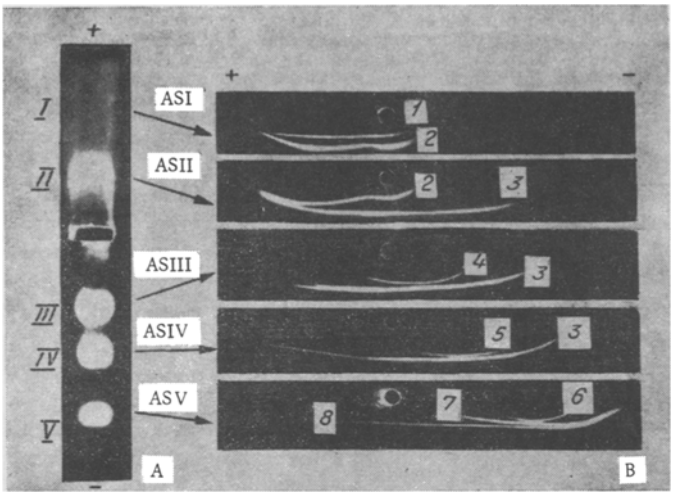

Fig. 1. Immunoelectrophoretic spectrum of tissue antigens of the iris detected by antisera against five fractions of extract of hen iris. A) Separation of extract of hen iris into five fractions by electrophoresis in agar gel; B) reaction of each antiserum with total extract of hen iris. ASI-ASV) antiserum against fractions I-V of the iris; 1-8) No. of antigens.

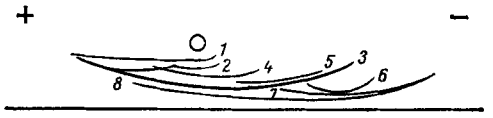

Fig. 2

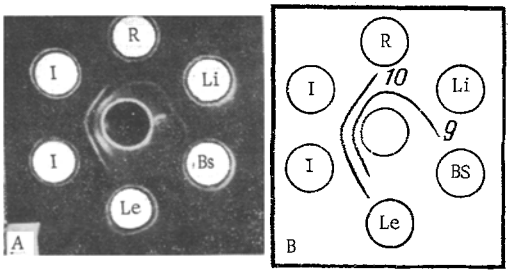

Fig. 3

Fig. 2. Composite spectrum of tissue antigens of iris: 1-8) No. of antigens.

Fig. 3. Identification of interorgan antigen (9) and organ-specific antigen of the iris (10) in Ouchterlony's test: A) photograph; B) diagram. Central well contains antiserum against fraction I of iris, exhausted with hen lens. Peripheral wells contain extracts from hens' organs: I) iris, R) retina, Li) liver, BS) blood serum, Le) lens.

EXPERIMENTAL RESULTS

By immunoelectrophoresis three serum and eight tissue antigens were found in the hen iris. The serum antigens – an albumin and two antigens with mobility corresponding to γ-globulins – were detected in the iris with the aid of antiserum against hen serum. Antisera obtained against five fractions of the iris revealed mainly tissue antigens. Each of the antisera against five fractions of hen iris formed two or three clearly distinguishable precipitation bands in the immunoelectrophoresis test with a total extract of the iris (Fig. 1B). Tissue specificity of the iris antigens was determined from the results of tests with each antiserum against extracts from the hen organs, and by disappearance of the corresponding precipitation arc from the antigenic spectrum of the iris on absorption of the antisera with the same extracts. From the results of this analysis a composite spectrum of the tissue antigens of the iris detectable immunoelectrophoretically was drawn up (Fig. 2) and their distribution in other organs of hens was determined (Table 1).

The results given in Table 1 were confirmed by Ouchterlony's test. In addition, because of the greater sensitivity of this method than of immunoelectrophoresis, antibodies against two other antigens were detected in antiserum against fraction I of the iris (Fig. 3). One of them was an interorgan antigen, the other specific for the iris only (Table 1).

TABLE 1. Organ Specificity of Tissue Antigens of Hen Iris

No. of antigen	Material tested: iris	retina	lens	brain	liver	muscle	Identification	Tests in which detected
1	+	+	+	+	+	+	Interorgan	IE
2	+	−	+	−	−	−	α crystallin	Same
3	+	+	+	−	−	−	β crystallin	»
4	+	−	+	−	−	−	δ crystallin	»
5	+	−	−	−	+	+	Interorgan	»
6	+	+	−	+	+	+	»	»
7	+	+	−	+	+	+	»	»
8	+	−	−	−	+	+	»	»
9	+	+	−	+	+	+	»	ID
10	+	−	−	−	−	−	Organ-specific iris antigen	Same

Legend +) Antigen present; −) antigen absent; IE) immunoelectrophoresis; ID) Ouchterlony's immunodiffusion test.

Previously two antigens, one characteristic of the iris and retina, the other for the retina, iris, and brain, were discovered in the hen retina [2]. With the aid of antiretinal sera, these antigens also were found in the hen iris. The absence of antibodies against them in antisera against the retina can be explained by the relatively small quantity of these antigens found in the iris compared with the retina in hens.

Altogether, therefore, three serum and 12 tissue antigens were found in the hen iris. The presence of serum antigens in the hen iris has been described by Bours [6]. As regards the tissue antigens of the iris and their specificity, no special study of this problem has hitherto been made. The presence of a tissue-specific antigen in the hen iris is mentioned in only one paper, but the question of the organ specificity of this antigen remains in doubt, for the authors concerned showed its absence in other tissues only in relation to the blood serum and lens [9]. However, antigens Nos. 7, 8, and 9 revealed by the present experiments (Table 1) also were absent in the lens and blood serum of hens, but they were not specific for the iris.

Antigen No. 8, found only in the iris and muscles, is particularly interesting. On immunoelectrophoresis with lyophilized extract from iris and muscles this antigen formed a characteristic long precipitation band (Fig. 1B), whereas in the reaction with freshly prepared extract of iris it formed a short arc, situated far toward the cathode in the antigenic spectrum of the iris. This antigen is probably specific for muscle cells as a whole and reflects the muscle tissue present in the iris. Its further analysis could be interesting in connection with views expressed in the literature on the special neural origin of the muscles of the iris [11].

Besides the tissue antigens described above, a complete assortment of antigens specific for the lens, namely α, β, and Δ crystallins, was discovered in the hen iris, in agreement with results obtained by other workers [6, 8, 9]. However, the synthesis of crystallins is a specific feature of the cells of the lens alone, and the functional significance of the fact that these proteins were also found in the iris is not clear. It is also not known whether crystallins were synthesized in the iris or whether they diffuse into it from the lens. This latter hypothesis is a very likely one because of the close morphological contact between the lens and the iris at all stages of ontogeny.

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