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Immunochemical Studies of Chick Iris

S. K. Brahma, J. Bours and W. J. van Doorenmaalen

Department of Medical Anatomy and Embryology, The State University of Utrecht, Janskerkhof 3A, Utrecht, The Netherlands

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Immunochemical properties of chick iris were investigated by various methods. It appears from these studies that chick iris contains antigens which are immunologically identical to all the antigens of the adult lens.

1. Introduction

The occurrence of lens antigens in extra-lenticular eye tissue has been recorded by a number of workers (see Clayton, Campbell and Truman, 1968). According to Langman and Prescott (1959), Maisel and Langman (1961) and Maisel and Harmison (1963a, b) chick iris contains antigens that are immunologically identical to the lens antigens. Clayton et al. (1968), like Maisel and Harmison (1963, a, b), and van Doorenmaalen (1964), have shown that chick iris contains proteins with the antigenic properties of lens α-crystallin. According to Clayton et al. (1968), several of the antigenic components of the chick lens "long line" are also present at a low concentration in iris. Zwaan (1963, 1968), on the other hand, reported that detection of lens antigens in iris is only a result of post-mortem diffusion from the lens to the iris tissue.

The purpose of the experiments reported here was to reinvestigate immunological properties of chick iris, and to compare them with those of chick lens antigens by various immunochemical methods.

2. Materials and Methods

The iris tissue used throughout the experiments was dissected carefully from chicken heads, which were collected immediately after decapitation from the slaughterhouse and transported to the laboratory under ice. These were washed and homogenized in cold distilled water and then centrifuged at 49,000 **g** for 30 min at 4°C. The supernatant was then carefully removed, lyophilized, and stored at −20°C until needed. Chick lenses dissected from the same materials were also processed in the same way as reported here for the iris.

With these two lyophilized samples the following experiments were carried out.

Series A

Four rabbits from 1 litter were made immunologically unresponsive to iris extract by injecting iris extract (100 mg/ml in saline) intraperitoneally into these new born animals within 24 hr after birth. When the litter was 6 weeks old, all the members were injected subcutaneously at 4 different sites on the back with chick lens extract (8 mg/0·5 ml in saline incorporated in an equal volume of Freund's adjuvant). This was repeated 3 times at an interval of 2 weeks. 10 days after the last injection sera were collected from the marginal ear veins and tested against iris and lens extracts my micro-immunoelectrophoresis according to Scheideggar (1955) in High Resolution Buffer, pH 8·9 (LKB A.B.)

Series B

In this series, 7 rabbits out of 2 separate litters were made immunologically unresponsive to lens antigens by injecting lens extract (100 mg/ml in saline) intraperitoneally into the newborn rabbits, as with the previous series, followed by injections of iris extract (8 mg/ 0·5 ml in saline incorporated in an equal volume of Freund's adjuvant) subcutaneously at 4 different sites on the back of the animals. The same schedule was followed as in series A and the collected sera were tested against iris and lens antigens by micro-immunoelectrophoresis.

Series C. Isoelectric focusing of lens and iris extracts

Iris and lens extracts were subjected to isoelectric focusing in flat acrylamide gel plates, with some modifications (Bours and van Dooremaalen, 1970) of the method of Awdeh, Williamson and Askonas (1968). 5% acrylamide gel slabs of 1-mm thickness were prepared with a final concentration of 2% "Ampholine" carrier ampholytes (LKB-Produkter, A.B., Sweden) covering a pH range from 3 to 10. 3 mg of iris and 1 mg of lens lyophilized samples were dissolved in 50 μl distilled water and were soaked in 1 cm² Whatman No. 3 MM filter paper. These were placed on the gel slab 30 mm away from the anodic side and kept under a constant current of 4 mA. An increasing voltage from 45 to 350 V was applied and the experiment was carried out in a humid chamber at room temperature. After the run, the established pH gradient in the gel was determined with a flat membrane glass electrode (Radiometer, type G 242C). Gels were then washed extensively for several days with trichloroacetic acid (TCA) at different concentrations starting with a 10% and ending in a 3% solution. Gels were then stained for 2 hr with 0·5% Coomassie Brilliant Blue R-250 (Serva) in a mixture of methanol–acetic acid–water (45:9:46) followed by washing in the same solvent until the background appeared to be colourless. The gels were then photographed.

Series D

Iris and lens extracts were tested by micro-immunoelectrophoresis against anti-total lens protein, anti- α-crystallin, and anti-FISC (first important soluble crystallin, Rabaey, 1962) sera. The anti- α-crystallin and anti-FISC sera were from the experiments reported earlier (van Doorenmaalen, Brahma and Hoenders, 1968).

Series E

Iris and lens extracts were tested by the two-dimensional crossed-electrophoresis technique of Laurell (1965) and Clarke and Freeman (1968), with some modifications introduced by Clayton, Campbell and Truman during the "International Working Party on Lens Crystallins," organized by the group in Edinburgh, 1970.

3. Results

When the tolerant sera from rabbits of the first two series were tested by immunoelectrophoresis against both iris and lens extracts, no precipitin line could be seen.

Isoelectric focusing of lens and iris extracts shown in Plate 1 reveals that the patterns of focused bands in the two tissues are nearly the same, differing primarily in the FISC region of lens. It appears that the lens FISC-group contains 7 to 8 focused bands with different isoelectric points, while the corresponding region in iris has less than half this number.

Plate 2(a) and (b) shows immunoelectrophoretic patterns obtained when lens and iris extracts were tested against chick anti-total lens protein serum. The resemblance

of the antigenic determinants of the two tissues is clearly visible. This was also revealed when iris and lens were tested with anti-FISC [Plate 2(c) and (d)], and anti-α-crystallin sera by immunoelectrophoresis [Plate 3(b) and (c)]. The precipitin band, developed when iris was tested against anti-FISC serum by immunoelectrophoresis, was wide and faint, and did not have a typical FISC-like appearance of the arc which one gets when lens extract is treated with anti-total lens protein, or anti-FISC sera [Plate 2(a) and (d)].

The two-dimensional crossed-electrophoresis of iris extract and anti-FISC serum produced a short and weak precipitin band, in comparison to the band obtained by crossed electrophoresis of lens extract and anti-FISC serum [Plate 4(a) and (b)].

4. Discussion

Clayton, Campbell and Truman (1968) reported that substances with the antigenic specificities of α-crystallin and long line are present in low concentration in iris. Our results confirm this; they have immunological determinants identical to those of the lens α-crystallin and long line. The said authors could not detect β-mobility components (FISC) in chick iris. We, on the other hand, like Maisel and Harmison (1963a), found that some components of the β-group (FISC) are present in iris and carry the same immunological determinants. These observations seem to explain why we failed to detect any precipitation band in the first two series (A & B) of our experiments where immunological tolerance was induced in neo-natal rabbits for iris and lens respectively, followed by a challenge either with lens or iris extracts. It also appears from the results of series B that iris does not contain any specific antigen, as reported by Maisel and Harmison (1963a). The shape of the precipitin curve of the FISC, obtained when iris was tested by immunoelectrophoresis against anti-total lens protein serum [Plate 2(a) and (b)], can be explained as follows: since iris showed a smaller number of focused bands in the FISC-group (Plate 1) as revealed from isoelectric focusing experiments, it probably contains a smaller number of antigenic constituents. When these constituents diffuse from the point of migration, on the basis of their electrophoretic mobility, and then precipitate with the antibody, they give rise to an arc wider and fainter than lens-FISC; and this antigenic group could only be clearly distinguished when tested against anti-FISC serum [Plate 2(c) and (d)]. This, together with the results obtained from quantitative two-dimensional crossed-electrophoresis [Plate 4(a) and (b)], show, as did Maisel and Harmison (1963a) that the concentration of these components of the FISC-group is rather low in iris.

It appears from the different experiments, reported here, that iris contains a number of antigenic determinants which have the same immunological properties as those of the lens. It has also been shown that the number of focused bands of the FISC-group in iris is less than in lens, and also that iris does not have any tissue-specific antigen.

A possible leakage of these antigens from the lens to the iris, as suggested by Zwaan (1963, 1968), could be ruled out by the fact that time between the collection of chicken heads and the dissection of iris was very little. Moreover, had there been a diffusion we could have expected all the components of lens FISC in the iris since it was found that in the 12-week-old lens most of the FISC antigen is superficially located (Brahma and van Doorenmaalen, in preparation) like the α and β crystallins (Zwaan, 1968).

Clayton, Campbell and Truman (1968) also do not support the leakage hypothesis

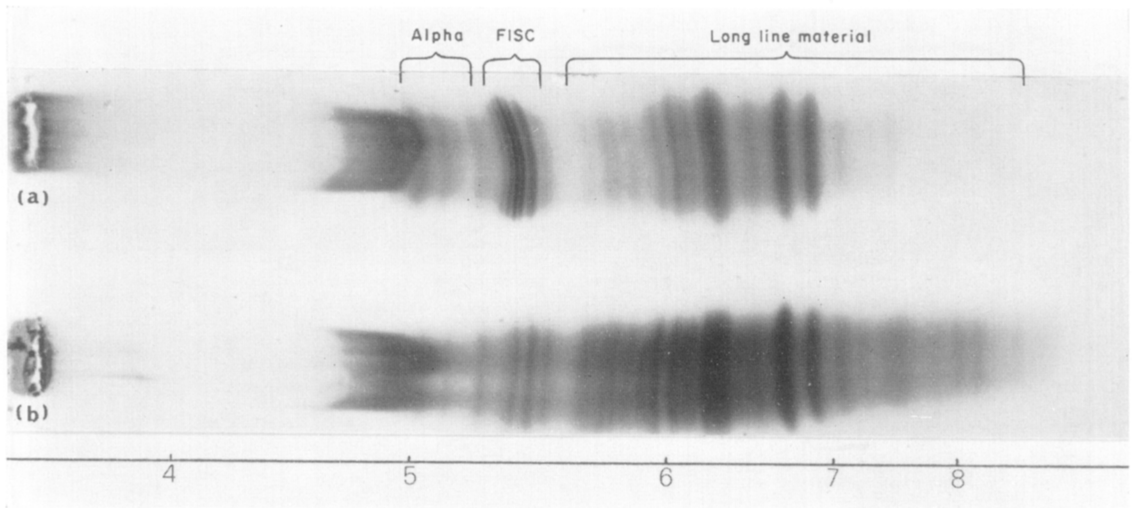

PLATE 1. Isoelectric focusing of (a) 1 mg chick lens and (b) 3 mg iris extracts in a 5% polacrylamide gel containing 2% "Ampholine" carrier ampholytes (LKB-Produkter, A.B., Stockholm, Sweden) in a pH range from 3 to 10. The experiment was done at room temperature, with an increasing potential difference starting from 45 to 350 V, and at a constant current of 4 mA. The scale shows the pH values along the gel. The number of focused bands in the FISC region appears to be less in the iris.

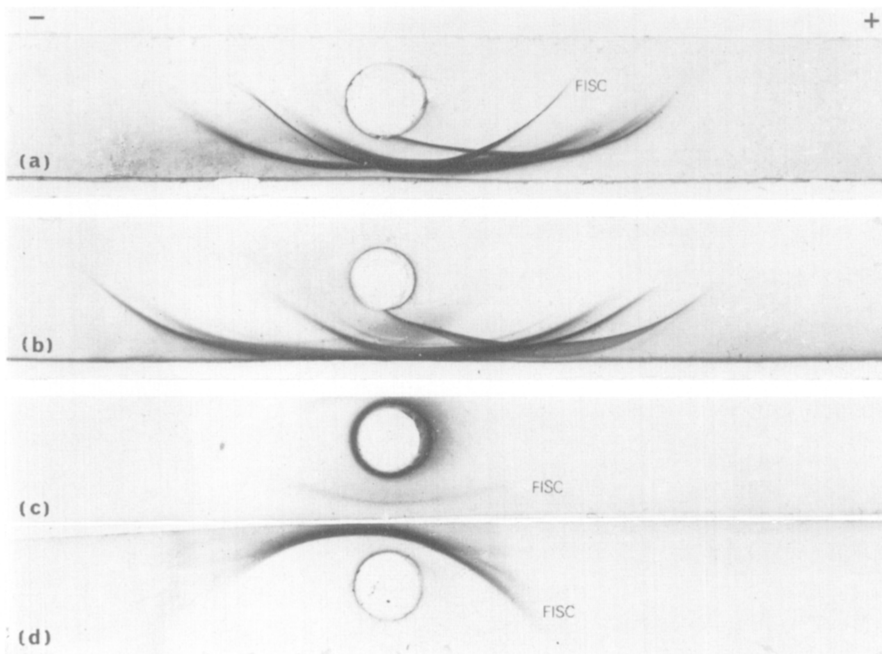

PLATE 2. Immunoelectrophoretic analysis of (a) chick lens and (b) chick iris extracts tested with chick anti-total lens protein serum. The iris extract formed precipitin bands which corresponded to those of lens proteins, except the FISC-line which was not visible in iris as such. With (c) iris extract and (d) lens extract the immunoelectrophoretic analysis against anti-FISC serum showed the presence of the FISC-antigen in the iris tissue.

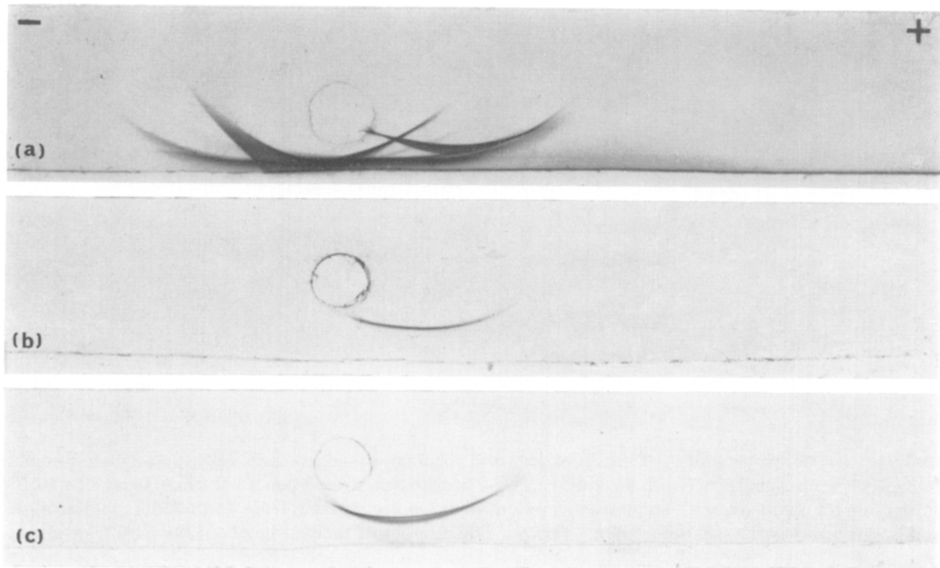

PLATE 3. Immunoelectrophoretic analysis of (b) chick iris and (c) chick lens extracts tested with anti- α-crystallin serum. A single precipitin band located at the anodic side was formed with iris and lens extracts. The precipitin bands of the lens–anti-lens system in (a) serve as a control.

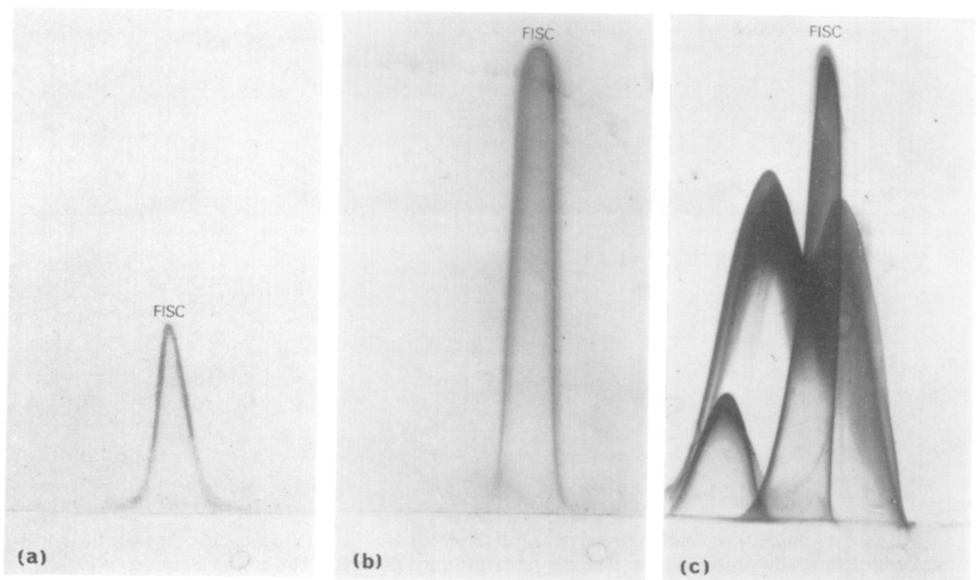

PLATE 4. 2D crossed-electrophoresis in 1% agarose of (a) chick iris and (b) chick lens extract tested with anti-FISC serum. A single precipitin curve could be observed in the region of the FISC. The precipitin curves with lens extract and lens anti-total crystallin serum serve as a control (c).

of Zwaan (1963, 1968) to explain the existence of lens protein antigens in the retina and iris.

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