IMMUNOELECTROPHORETIC ANALYSIS OF THE LENS IN THE POSTNATAL DEVELOPMENT OF MICE

V. M. Barabanov

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In the individual development of the vertebrates part of the ectoderm of the embryo's head differentiates into the lens under the influence of the presumptive retina. It has now been shown reasonably clearly that antigens characteristic of the definitive lens are formed mainly during the period of embryonic development of the lens [4, 8]. Meanwhile, the internal antigenic differentiation of the lens has not yet been adequately investigated. The lens of adult animals contains structures differing in their morphology: the capsule, the lenticular epithelium, the cortex and nucleus of the lens. It might be expected that the antigenic properties of these structures are different. In fact, it has been found that the cortex and nucleus of the crystalline lens of hens differ in their antigenic composition [10]. Antigenic differences between the epithelium and the fibrous structures of the lens have been demonstrated in the ox and triton [6]. In particular, it has been found that the α-crystallins of the epithelium, the cortex, and the nucleus of the bovine lens differ in their electrophoretic mobility [9].

Investigation of the antigens of the mouse lens in embryogenesis have shown that the electrophoretic properties of one of the antigens of the α-crystallin fraction in adult animals differ from the properties of this antigen is embryonic and newborn mice [1].

In the present investigation the formation of the antigens of the α-crystallin fraction was studied in the postnatal development of mice.

EXPERIMENTAL METHOD

Antisera were obtained for the experiments against lenticular antigens of mice and frogs. Rabbits were immunized for 3 months in accordance with a scheme including both intravenous and subcutaneous injection of the extracts mixed with Freund's adjuvant [2]. The titer of the antisera was determined by the ring-precipitation reaction. In two sera (Nos. 14 and 14a) obtained against the lenticular antigens of mice, it was not below 1:1000. In immunoelectrophoresis both sera gave a clear reaction with one of the antigens of the α-crystallin fraction of the mouse lens, while one of them (serum No. 14) contained antibodies almost entirely against this antigen. The titer of serum No. 10a against the frogs' lens was 1:80,000; in immunoelectrophoresis it formed precipitation bands with the α- and β-crystallins of the mouse lens.

The antisera obtained in this manner were used to investigate the lenses of noninbred mice at different stages of postnatal development. The lenses were extracted, the capsule moved, and extracts made in tris-buffer (pH 8.5); M = 0.1), the ratio between tissue and buffer being 1:10. In addition, the lenses of adult mice and mice aged 10, 14, and 21 days were separated into cortical and nuclear zones and corresponding extracts made from the separated tissues. The cortex and nucleus of the lenses of adult rats, rabbits, and guinea pigs were investigated in the same way.

The extracts were analyzed by immunoelectrophoresis in an agar gel using the apparatus described previously [3]. The agar (1.25%) was made up in tris-buffer (pH 8.5; M = 0.1) and the electrode compartments of the apparatus were filled with this same buffer. Electrophoresis continued usually for 75-90 min with a potential gradient of 7.5-6.5 V/cm. Gutters cut out of the agar parallel to the axis of migration of the antigens were filled with antisera, and the plates were placed in a humid chamber at 4°. The results of the reactions were read next day.

EXPERIMENTAL RESULTS

The results of the investigation of the lens extracts of the mice in the postnatal period are given in Fig. 1. The antigen of the α-crystallin fraction from the adult mice gave two concentration maxima during electrophoresis:

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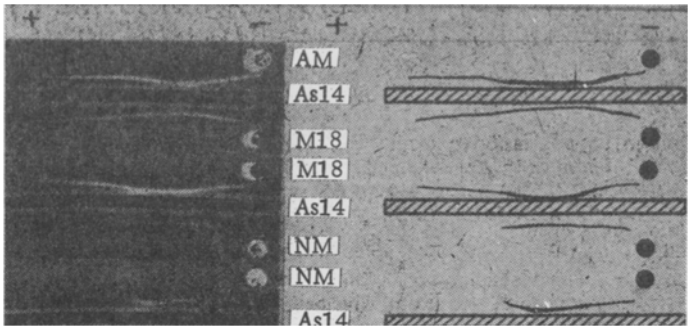

Fig. 1. α-Crystallins of lenses of newborn, 18-day, and adult (3-month) mice. AM – Extract from lenses of adult mice. M18 – extract of lenses from 18-day mice; NM – extract of lenses from newborn mice; As 14 – rabbit antiserum No. 14 against lenses of adult mice.

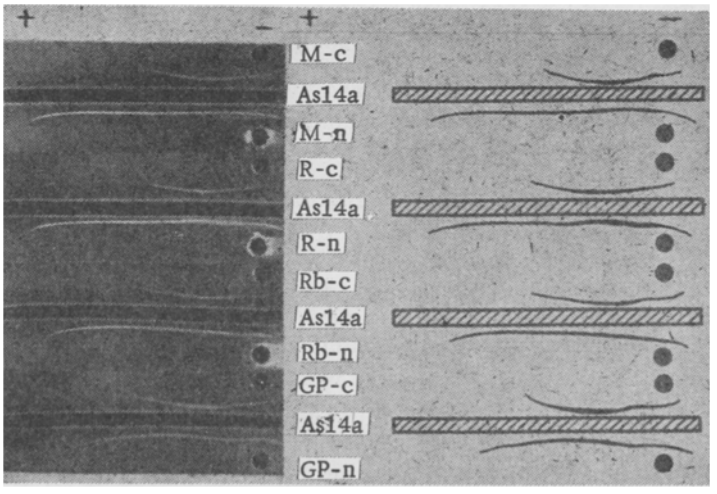

Fig. 2. α-Crystallins of the cortex and nucleus of lenses of adult mice, rats, rabbits, and guinea pigs. M-c and M-n – extracts from cortex and nucleus of lenses of mice, R-c and R-n – extracts from cortex and nucleus of lenses of rats, Rb-c and Rb-n – extracts from cortex and nucleus of lenses of rabbits, GP-c and GP-n – extracts from cortex and nucleus of lenses of guinea pigs, As 14a – rabbit antiserum No. 14a against lenses of adult mice.

the first corresponded to α-crystallins with high mobility and the second to α-crystallins with lower mobility. The lenses of the newborn mice contained only α-crystallins giving the second concentration maximum on immunoelectrophoresis and none of the subfraction of antigen with higher mobility. Judging by the length of the precipitation band formed in the reaction with extracts from the lenses of 18-day mice, by this period of postnatal development the subfraction of highly mobile α-crystallins had already appeared.

Such marked differences in the composition of the antigens of the α-crystallin fraction were observed not only on comparing the lenses of mice of different ages but also on investigating extracts from the cortex and nucleus of the lenses of adult mice and certain other animals by immunoelectrophoresis. Immune sera Nos. 14a and 10a were used to investigate extracts from the cortex and nucleus of the lenses of adult mice, rats, rabbits, and guinea pigs. The results given in Fig. 2 show that the mobility of the analogous antigens of the α-crystallin fraction, and the position and shape of the precipitation bands formed by them differed in their fine details in the different species. However, in all the animals investigated, including mice, only α-crystallins giving the second concentration maximum in immunoelectrophoresis were found in the cortex of the lenses, whereas in the nucleus of the lenses the

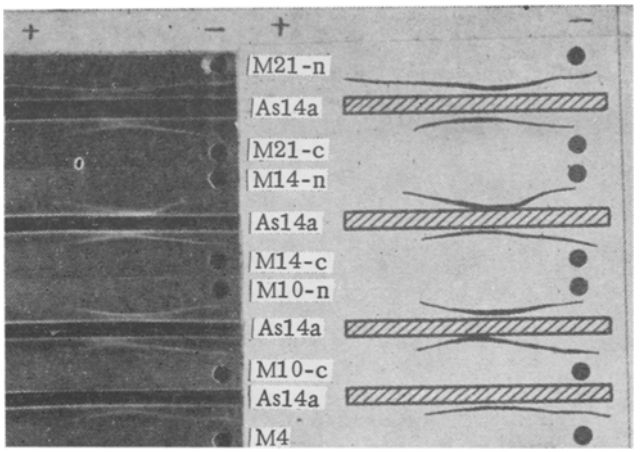

Fig. 3. α-Crystallins of the cortex and nucleus of mouse lenses in the postnatal period. M21-c and M21-n – extracts from the cortex and nucleus of nucleus of lenses of 21-day mice; M14-c and M14-n – extracts from the cortex and nucleus of lenses of 14-day mice; M10-c and M10-n – extracts from the cortex and nucleus of lenses of 10-day mice; As 14a – rabbit antiserum No. 14a against the lenses of adult mice; M4 – extract from the lenses of 4-day mice.

subfraction of antigen with higher mobility corresponding to the first concentration maximum of antigen was found in addition to this fraction. Comparison of these results with those given in Fig. 1 shows that the position of the precipitation band formed in the zone of the α-crystallins by the extract from the lenses of the adult mice was largely determined by the proteins of the lens nucleus. Meanwhile the position of the precipitation band formed by antigen from the lens cortex of the adult mice corresponded more closely to the position of the precipitation bands revealed during immunoelectrophoresis of extracts from the lenses of newborn mice.

The differences in the composition of the α-crystallins observed during analysis of extracts from the lenses of newborn and adult mice, and also from the cortex and nucleus of the lenses of adult mice may indicate that the definitive formation of the nucleus of the lens takes place in the postnatal development of these animals. This is also shown by macroscopic analysis of the lenses when removed from mice of different ages. The nuclei of the lenses extracted from the newborn mice and also from mice aged 3, 6, and 10 days, quickly became opaque, whereas the lenses extracted from the adult mice remained transparent.

To test this hypothesis, extracts from the cortex and nucleus of the lenses of mice aged 10, 14, and 21 days were investigated by means of antisera Nos. 14a, and 10a. The results of immunoelectrophoresis carried out with serum No. 14a are shown in Fig. 3. It is clear from Fig. 3, that both in the cortex and in the nucleus of the lenses of the 10- and 14-day mice the antigens belonging to the α-crystallins were characterized by the same mobility, analogous to the mobility of the antigen from the lenses of the 4-day animals. Meanwhile, in the nucleus of the lens of the mice on the 21st day of life a subfraction of antigen with higher mobility appeared, as a result of which the precipitation band formed by this antigen extended much farther toward the anode than the precipitation band of the antigen from the lens cortex of the mice of the same age. In the experiments with the serum against the frogs' lenses (No. 10a) similar results were obtained.

The specificity of the antigens of the α-crystallin fraction from the cortex and nucleus of the lenses of the adult and 10-day mice was investigated by immunoelectrophoresis with absorbed sera and in Ouchterlony's double diffusion reaction. Analysis of the extracts from the lens nucleus of the adult mice by immunoelectrophoresis showed that after absorption of the sera with antigens from the lens cortex, none of the precipitation bands observed in the reactions with unabsorbed sera were formed in the agar. In Ouchterlony's reactions the extracts from the cortex and nucleus of the lenses of adult and 10-day mice formed precipitation bands which merged with each other without forming "spurs". The results of these analyses thus demonstrated the serological identity of the antigens of the α-crystallin fraction from the cortex and nucleus of the lenses of mice in the late and early stages of development.

The results described above show that differentiation of the lens tissue, which begins in embryogenesis, also continues in the postnatal development of mice. At the time when the young mice open their eyes and the lens begins to perform its definitive function, the nucleus of the lens is evidently finally formed. At the same time the antigens of the lens nucleus belonging to the α-crystallins probably undergo structural changes leading to the appearance of a subfraction with higher electrophoretic mobility. The change in the electrophoretic mobility of the α-crystallins in ontogenesis of cows discovered by Bon [5] by means of electrophoresis on paper, and regarded by him as a sign of recapitulation, may possibly be of similar nature.

These results, and also those obtained by other authors [10], demonstrate that the internal antigenic differentiation of the lens in individual development is manifested not only by the formation of new antigens [7], but also by changes in the relative concentration of the individual antigens and, in some cases, with changes in their physical properties also.

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