FORMATION OF ORGAN-SPECIFIC ANTIGENS OF THE LENS IN ONTOGENESIS IN MICE

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The supplementing of morphological by immunological methods in embryology gives a more complete picture of the processes of differentiation of organs and tissues. The appearance of new antigens possibly reflects the specific adaptation of biosynthesis. In this case the study of antigen formation may provide the basis for the elucidation of the mechanisms responsible for tissue differentiation and specialization. In the first place, however, it is essential to have some idea of the dynamics of the changes in the antigenic structure in the course of organogenesis. Many investigations conducted on the lens of birds and amphibians have been devoted to this problem [3, 5, 6, 8, 9, 11, 13-15].

Meanwhile, hardly anything is known of the development of the lens antigens in mammalian ontogenesis. The published investigations of the localization of the lens antigens in mouse embryos [7] and of the antigenic composition of the lens of newborn rabbits [10] contain no systematic analysis of the lens antigens from the moment of appearance of the anlage of this organ until the time of its final formation.

The object of the present investigation was to study the organ-specific antigens of the lens in ontogenesis in noninbred mice.

EXPERIMENTAL METHOD

Antisera from four rabbits were used in the experiments: 3 antisera against the antigens of the lens of adult mice and 1 against the lens antigens of newborn mice. The antisera were obtained as a result of prolonged immunization (from 3 to 12 months), for the water-soluble lens proteins of mice possess low antigenicity for the rabbit. The scheme of immunization was described previously [1]. The titer of antisera was 1:20,000-1:40,000, and they possessed adequately high organ specificity. With extracts of striated muscles, heart, kidney, and spleen, and also with the blood serum of adult mice the antisera did not react, but if the sera were concentrated 2-3 times by ultrafiltration, turbidity of the agar was observed in the reaction with mouse liver extract.

As antigens, extracts from lenses taken from mice in different stages of ontogenesis were used. Extracts were investigated from the invaginating placodes of 10-day embryos, from anlagen of the eyes of 11-day embryos, from the lenses of 12, 13, 15, 16, and 18-day embryos, and also from the lenses of newborn mice and mice sacrificed 10 and 60 days after birth. All the extracts were prepared in 1/15 M phosphate buffer, pH 7.7, the ratio of tissue to buffer being 1:10.

The organ-specific antigens of the lens were investigated by means of the double diffusion reaction in agar plates by Ouchterlony's method. Preliminary experiments showed that, in contrast to the lens antigens of the lower vertebrates, the water-soluble antigens of the lens of certain rodents (mice, rats, guinea pigs) react with the corresponding antisera in buffered agar, pH 7.4-8.5, at 4-20°. In the case of deviation from these conditions, the antigens diffusing into the agar form a wide zone of turbidity around the wells.

In the course of the investigation cross reactions were carried out, in which whole extracts of the lenses of mice of different ages and whole antisera were used. In addition, extracts of lenses of animals of each period of development studied were titrated by the method described by G. I. Abelev [2]. In the last case one of the peripheral wells cut out of the agar was filled with whole extract, and the others with extract in dilutions of 1:4, 1:16, 1:64, 1:256, and 1:1024. Whole antiserum was poured into the central well.

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Number of Precipitation Lines in Reaction with Extracts from Lenses of Mice of Different Ages*

Antiserum	Age of mice (in days)									
	antenatal period							postnatal period (counted from the day of birth)		
	10		12	13	15	16	18	1-2 (newborn)	8-10	60 (adult)
Against lenses of adult mice (No. 2944)	–	Turbidity of agar	–	4	4	4	4	4	4	4
Against lenses of newborn mice (No. 2951)	–	"	–	4	4	4	4	4	4	4
Against lenses of adult mice (Nos. 2955 and 2 mixed)	0	"	2	–	2	–	2	2	–	2

* The results of typical experiments are given. Legend: –) no reaction performed; 0) negative reaction.

EXPERIMENTAL RESULTS

The results obtained by the study of the process of formation of the organ-specific lens antigens during ontogenesis of mice, by means of the cross reactions, are given in the table.

As the table shows, the clearest reaction was observed with antisera Nos. 2944 and 2951, obtained against extracts from the lenses of adult and newborn mice respectively. The reaction with the mixture of two other sera (Nos. 2955 and 2) against the lens of the adult mice was less marked. As a result of the reaction of all the antisera with extracts from the anlagen of the eyes of the 11-day embryos, only turbidity of the agar was observed. Antisera Nos. 2944 and 2951 reacted with extracts from the lenses of the mice during the postnatal period with the formation of 4 precipitation lines, whereas the mixture of two other antisera gave only 2 precipitation lines.

The results described show that during comparison of the antigenic composition of the lens of adult and newborn mice, no additional antigens characterizing this stage of development (i.e., stage-specific antigens) were found in the lens of the newborn mice. Antiserum against the lens of the newborn mice reacted with the water-soluble antigens of the definitive lens with the formation of 4 precipitation lines, i.e., just as in the homologous reaction with extract from the lenses of the newborn mice.

The results of the reactions with the embryonic lenses showed that the organ-specific antigens of the definitive lens appear for a quite short period in the embryogenesis of mice. Whereas the extract prepared from the anlagen of the eyes of 11-day embryos formed only slight turbidity in the agar with sera Nos. 2944 and 2951, the water-soluble antigens of the 13-day lens formed 3 clear precipitation lines, and if the reaction in agar was continued for longer, a 4th line appeared (Fig. 1). Similar results were obtained with the other two antisera (Nos. 2955 and 2), the only difference being that these antisera formed fewer precipitation lines than the preceding sera. Lens antigens were found on the 11th-12th days of embryonic development of the mice, and could not be found in the extract of the invaginating placodes of the 10-day embryos.

As stated above, the results obtained when the reactions were carried out with whole extracts are given in the table. However, in Ouchterlony's reaction some of the precipitation lines may be complex, formed of several lines situated in the same narrow zone of agar. To determine the maximal number of antigens revealed by these antisera, the reactions were carried out with serial dilutions of extracts, i.e., titration reactions. For instance, in the reaction with sera Nos. 2944 and 2951, in which extracts of the lenses of adult and newborn mice were titrated, as many as 7 precipitation lines were observed. The results of analogous experiments carried out with embryonic lenses and with serum No. 2951 showed that all the antigens detected in the lens of the adult and newborn mice were also detected in the lens of the 13-day embryos.

The agar reaction with serial dilution of the extracts may also be used for determining changes in the relative concentration of individual antigens in the process of development, if the final dilutions of the extracts are counted at which a particular precipitation line is still visible. On the basis of the results of three series of experiments in which extracts from lenses at different times of development and antisera Nos. 2944 and 2951 were used,

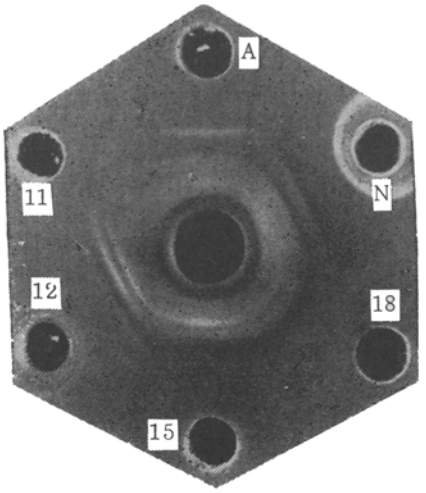

Fig. 1. Reaction of extracts of lenses of mice of different ages with antilens serum No. 2944; A, N, 18, 15, 13, 11) extracts from the lenses of adult, newborn mice, and mouse embryos of 18, 15, 13, and 11 days of development.

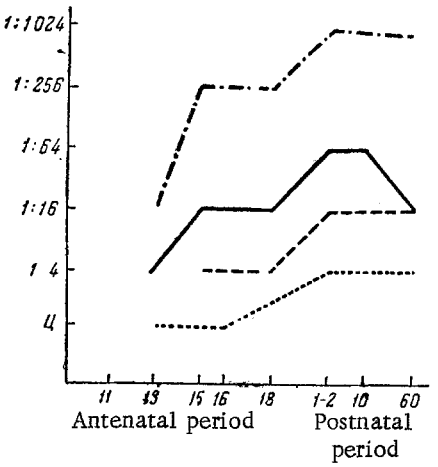

Fig. 2. Changes in the concentration of the four principal organ-specific lens antigens in the course of development in mice. Along the axis of abscissas—age of mice (in days); along the axis of ordinates—dilution of extracts.

a graph was plotted (Fig. 2), showing the changes in the concentration of the 4 principal lens antigens in the ontogenesis of the mice.

The results described demonstrate primarily the progressive increase in the concentration of the individual antigens from the moment of their appearance in the lens. Starting from the 18th day of intrauterine development, i.e., in the period preceding birth, the concentration of all the organ-specific antigens increased. These results are in agreement with results reported in the literature, obtained by investigation of the lens proteins of rat embryos [12].

The development of the antigenic structure of the mouse lens evidently continues in the postnatal period. For instance, the lens of mice 8-10 days old is similar as regards both the number of its antigens and their relative concentration to the lens of newborn animals, but later a decrease is observed in the concentration of one of the antigens (see Fig. 2).

The results demonstrate that the organ-specific antigens characteristic of the definitive lens arise in the embryogenesis of mice on the 11th-13th day of development. In this period intensive morphological transformations take place in the developing lens, as a result of which the organ acquires its definitive features [4]. With the further development of the lens the concentration of the principal organ-specific antigens progressively increases, especially in the period preceding birth. The formation of the antigenic structure of the lens evidently continues in the postnatal period, as shown by the decrease in the concentration of one of the antigens.

The present results differ from those of the investigation of the development of organ-specific antigens in birds. In particular, no organ-specific antigens could be detected in the mouse embryos on the 10th day of intrauterine development – in the period of invagination of the lens placode and of separation of the lens vesicle.

SUMMARY

The reaction of double diffusion in agar after Outcherlony was used to study the organ-specific antigens of the lens in the ontogenesis of noninbred mice. Crystalline antigens were discovered in embryos on the 11th-13th day of the development. Later the concentration of organ-specific antigens increased progressively, particularly in the antenatal period.

It is supposed that the formation of the antigenic structure of the crystalline lens continues also in the postnatal period, because it has been found that concentration of one of the antigens becomes less in mice older than 10 days.

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