SPECIFIC ANTIGENS OF THE LENS IN EYE RUDIMENTS OF CHICK EMBRYOS

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By now a need has developed to study the antigenic properties of tissues at the early stages of development. Such investigations may perhaps lead us nearer to an understanding of the mechanisms of organogenesis [1]. In studying organ antigens, and in particular the antigens of the lens, the first point to be determined is the time at which they develop during the course of embryogenesis. The data in the literature are contradictory. Some authors [4] maintain that organ specificity exists from the time that the organ is completely differentiated morphologically. Others [5, 6] have proposed that in the very first stages of development of the egg cell, and even in unfertilized ova the determinant groups specific to the lens are already present, and they appear to regulate the formation of organ antigens at later stages of development. However, most investigators [2, 3, 8] think that the appearance of antigens specific to the lens somewhat precedes the morphological differentiation of this organ.

Thus the question as to when antigens to the lens develop during embryogenesis cannot be regarded as solved. Several investigations along these lines have been undertaken principally with anti-lens sera from adult animals.

Only Burke et al., [4] has used sera against chick embryos incubated for 160 and 300 h. By means of these sera it was shown that at 96, 120, and 250 h incubation the lens antigens characteristic of the earlier stages (160 h incubation) were gradually replaced by other lens antigens characteristic of the later stages (300 h and above, or adult chicken).

The results of these authors showed that use of immune sera against embryonic lenses enabled the corresponding antigens against them to be indentified at earlier developmental stages.

The object of the present investigation has been to determine at what stage of chick embryo development antigens resembling lens antigens from 6- and 10-day-old chick embryos and adult birds could be identified.

METHOD

We obtained immune sera against lenses of 6-day-old chick embryos (from 4 rabbits), against 10-day-old chick embryos (from 2 rabbits), and against adult chick lenses (from 5 rabbits); the sera were obtained by immunization of the rabbits by means of Froind's adjuvant. Then the sera against the lenses, having a titer of 1:20,000 or more were absorbed on a powder prepared from chicken liver by the method proposed by Coons and Kaplan [7] in order to remove species-specific antibodies.

After twofold adsorbtion of the serum we tested for specificity by the ring-precipitation reaction and by precipitation in agar with normal chick serum and, then with extracts from various tissues—heart, liver, spleen, brain, adult chick ectoderm—and with extracts from the ectoderm, brain, and viscera of 6-day-old chick embryos (Fig. 1). After adsorption none of the sera reacted with any of the above mentioned antigens except the lens.

Altogether we carried out 4 sets of experiments. In series I, II, III and IV we used chick embryos at the stages of: 8-9 and 10-12, 14-15 and 16-18, 19-22 and 23-25, 27-30 and 33-36 pairs of somites respectively.

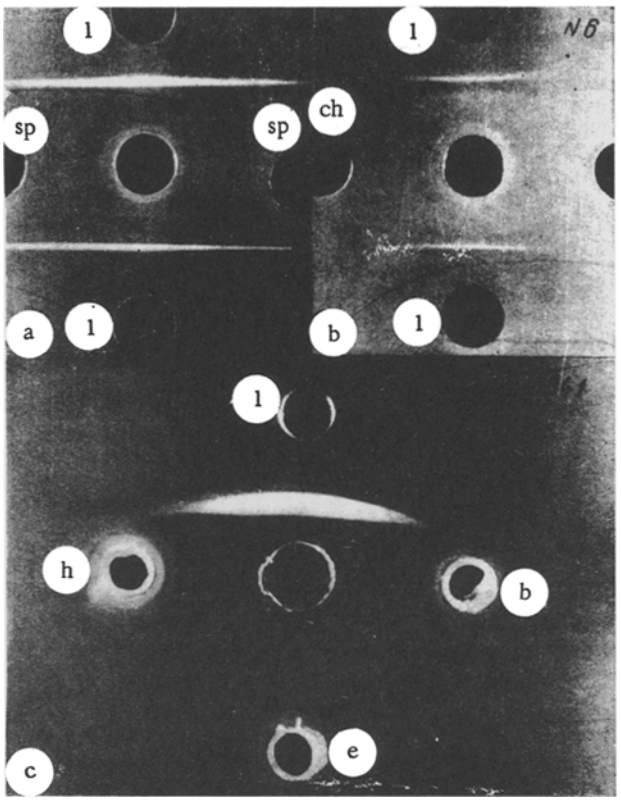

Fig. 1. Results of precipitation in agar on plates. In the central holes – sera against lenses of adult chicken (Nos. 14, 6) and against lenses of 6-day-old chick embryos (841); in the peripheral holes-normal blood serum of chicken (ch), extracts of various tissues from adult chicken, as follows: lens (1), heart (h), spleen (sp); also extracts of brain (b) and ectoderm (e) of 6-day-old chick embryo.

These stages were interesting for the following reason: in embryos with 7 pairs of somites the optic vesicles begin to form from the primitive forebrain, and then begin to increase in size. At the stage of 9 pairs of somites contact between the optic vesicles and the ectoderm begins. However, before the stage of 12 pairs of somites the presumptive lenticular ectoderm cannot yet be distinguished from the ectoderm of the head in regions not immediately adjacent to the optic vesicle.

In chick embryos at the stage of 14 pairs of somites, as a result of contact between the optic vesicle and the ectoderm, in the latter intracellular changes may be observed; the vacuoles disappear, and the nuclei lie perpendicular to the cell membrane which faces the optic vesicle. The cells become elongated. The initial stage of formation of the lenticular placode is observed.

At the stage of development of 19-22 and of 23-25 pairs of somites there is an invagination of the lenticular placode with formation of the optic vesicle, which however is still connected with the ectoderm.

In embryos at developmental stages of 27-30 and 33-36 pairs of somites the primary lenticular fibers may be observed to form.

For the experiment we took 250 chick embryos at each of the developmental periods mentioned above. In the I, II, and III series of experiments we used altogether 500, 950, and 750 embryos respectively.

On each occasion as antigen we used an aqueous saline extract prepared from tissue rudiments of the eyes of chick embryos at the developmental stages already mentioned (10 mg of tissue to 0.1 ml physiological saline).

The principal methods of investigations which we used were the ring precipitation test and precipitation in agar in capillaries and on plates.

Results of Ring Precipitation Tests between Sera Against Lenses of Chick Embryos of 6 and 10 Days Incubation and Lenses of Adult Chicken and Extracts of Tissue Rudiments of Eye of Chick Embryos at Various Stages of Development (8-36 pairs of somites)

Series of experiments	antigens-tissue rudiments of embryo eye (stage of development of somites)	No. of embryos	Sera against lenses (dilution 1 : 2)													
			6-day chick embryos					10-day chick embryos					Adult chicken				
			1:20	1:40	1:80	1:160	1:320	1:20	1:40	1:80	1:160	1:320	1:20	1:40	1:80	1:160	1:320
I	8—9	500	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	10—13		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
II	14—15	950	±	±	±	0	0	0	0	0	0	0	0	0	0	0	0
	16—18		+	+	+	0	0	0	0	0	0	0	0	0	0	0	0
III	19—22	950	+	+	+	0	0	+	0	0	0	0	+	0	0	0	0
	23—25		+	+	+	+	0	+	+	+	0	0	+	+	+	0	0
IV	27—30	750	+	+	+	+	0	+	+	+	+	0	+	+	+	+	±
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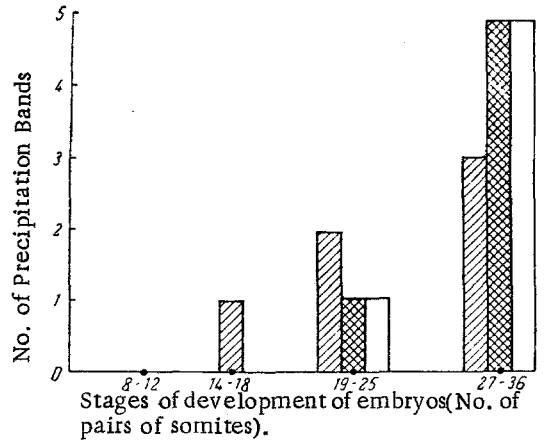

Fig. 2. Results of precipitation in agar in capillary tubes between anti-lens sera and extracts from tissue rudiments of eye of chick embryos. Oblique shading–experiments with sera against lenses of 6-day-old chick embryos; double shading–against lenses of 10-day-old chick embryos; unshaded–against lenses of adult chicken.

RESULTS

From the results given in the table it can be seen that extracts of tissue rudiments of eyes of chick embryos at the stage of 8-12 pairs of somites did not react with sera against lenses taken from 6- and 10-day-old chick embryos or from adult birds.

When as antigen we used extracts from embryos at the stage of 14-18 pairs of somites we obtained a positive reaction of sera against lenses of 6-day-old chick embryos, and a negative result with sera against lenses of 10-day-old embryos and adult chickens.

The reaction of extracts of tissues from more highly developed embryos at the stage of 19-22 pairs of somites was not restricted to sera against the 6-day-old chick embryos. The positive reaction (in a dilution of 1:20) was observed also with sera against 10-day-old embryos and against adult chickens.

As can be seen from the table as the chick embryos developed the intensity of the reaction with all these sera was enhanced.

The results of this experiment showed that even at the stage of 14-15 pairs of somites antigens are present in the tissue rudiments of the eyes of chick embryos, and these antigens resemble those obtained from the eyes of 6-day-old embryos.

At the later stages of development, from 19-22 pairs of somites onwards, other antigens also appeared which resembled those from the lens not only of 6-day-old embryos but also of 10-day-old embryos and adult chickens.

The results of the ring precipitation test completely confirmed those found by precipitation in agar in capillary tubes (Fig. 2).

By means of this reaction we also determined the number of antigens specific to the lens of 6- and 10-day-old chick embryos and of adult birds contained in the tissue rudiments of the eye of younger embryos. Thus in embryos having 14-18 pairs of somites there was one antigen resembling the antigen from the lens of a 6-day-old chick embryo; in an embryo with 19-25 pairs of somites 2 antigens were found specific to the 6-day-old lens, and one antigen was present specific to the 10-day-old embryo and to the adult animal; in an embryo with 27-36 pairs of somites there were 3 antigens resembling those from the lens of a 6-day-old embryo, and 5 resembling those from the lens of a 10-day-old embryo or adult bird.

The results of precipitation in agar confirmed those obtained by the ring precipitation tests.

Our results show that during the course of chick embryo development there is a regular change in the organ-specificity of the lens antigens: antigenic specificity characteristic of the lens of a 6-day-old embryo is present in the tissues of eye rudiments of chick embryos at earlier stages of development (14-16 pairs of somites) than is the antigenic specificity characteristic of the lenses of 10-day-old embryos (19-22 pairs of somites) or of adult chickens.

It can be seen that our results correspond to those of Burke et al., in that they indicate a definite sequence in the appearance of lens antigens during the course of embryogenesis: initially antigens are present which are characteristic of the earlier stages, and they are followed by others characteristic of the later stages. However, whereas Burke et al., found lens antigens at stages when the organ was completely differentiated morphologically, our results showed that lens antigens are present at the very earliest stages of formation before morphological differentiation has taken place.

From the results we may suppose that the use in similar experiments of immune sera against lenses at various stages of chick embryo development (for example 3 days) will enable us to find antigens specific to them at even earlier stages of embryogenesis.

We may hope that further study of organ-specific antigens at early developmental stages will lead to a better understanding of the relationship between the development of antigenic properties and morphological differentiation of the organ.

To work towards an understanding of the way organs are formed from their presumptive tissues it seems to us essential not only to make a study of the organ-specific antigens in the rudiment of the organ concerned but also to study the distribution of these antigens in other tissues. We will discuss this point more fully in a later work.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
