STUDY OF ANTIGENIC PROPERTIES OF ANIMAL TISSUES AND ORGANS IN ONTOGENESIS

INVESTIGATION OF SPECIES AND ORGANISM SPECIFICITY OF THE CRYSTALLINE LENS BY MEANS OF THE RING PRECIPITATION REACTION COMMUNICATION III*

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The serological reactions of precipitation and fixation of complement have been employed by many authors in the study of the antigenic structure of the crystalline lens of the eye in adult animals. It was found that the albumin of the crystalline lens in different animals possesses a high organism specificity. However, a number of authors [7, 10], on the basis of the cross reaction between crystalline lens antisera, and extracts of the crystalline lenses of different animals, have pointed out that the organism specificity of the crystalline lenses is only relative. The reactions between distant species of animals were very weak or not observable at all, while the associated species of animals gave positive reactions at high titers. On the basis of these findings, some authors [7] consider that the crystalline lens, together with organism specificity, must possess specific properties common to the species. More convincing data, suggesting a species specificity of the crystalline lens, by means of serological reactions has not been obtained.

Some investigators [9, 11] have found that crystalline lenses in embryos of man and hens possess a species specificity. Other investigations have been devoted to the study of the organism specificity of the antigens of the crystalline lenses of the chick and frog in embryogenesis [4, 5, 12]. The authors of these investigations, by using an identical precipitation reaction, obtained conflicting results. The contradictory nature of the findings has rendered difficult the conception of the orderly development of the antigenic organism specificity. The present investigation was undertaken for the purpose of studying this question.

EXPERIMENTAL METHODS

Taking into account the high degree of anticomplement nature of the embryonic tissues [5], and particularly of the crystalline lens [12], and also the fact that for the production of the complement-fixation reaction a considerably larger amount of the test substance is required than for the precipitation reaction, we decided in our experiments to use the ring precipitation reaction.

The antisera were obtained by immunizing four chinchilla rabbits (weighing 2.5-3 kg; females) with a saline extract of the crystalline lenses of adult ducks of the Peking variety, and also by the serum of these ducks according to the scheme described by P. N. Kosyakov [1]. The antisera of the crystalline lens of a duck were highly specific, and reacted with a homologous antigen at attenuations of 1:10,000, and 1:20,000. Tables 2-3 refer only to the results obtained with the serum, which gave a positive reaction at an antigen attenuation of 1:20,000.

* For Communication I see Byull. Eksptl. Biol. i Med. 1956, Vol. 41, No. 4. For Communication II see Byull. Eksptl. and Biol. Med. 1956, Vol. 41, No. 5.

It should be noted that the first attenuations of the antigen (1 : 50) were those small volumes of physiological solution (49) added to the tissue to form a saline extract. Subsequent attenuations were obtained by means of dilution with physiological solution. The immune sera against the serum of the duck reacted with the homologoud antigen attenuation of 1 : 30,000 and 1 : 50,000 (Table 1 refers only to the results obtained with the serum which gave a positive reaction on attenuation of the antigen at 1 : 50,000).

The sera were attenuated before use with an equal amount of physiological solution.

The tissue of the crystalline lens, heart and brain, used in order to obtain saline extracts, was freed from the adjacent tissues and cleansed of blood. The crystalline lens was separated from the crystalline capsule. In order to check that the blood had been thoroughly washed away, the washings were investigated. The saline extracts of the brain and heart served as control.

The reaction of ring precipitation was evaluated by the final attenuation of the antigen, and account was also taken of the degree of intensity of the ring, which was determined according to the following scale:

++++ heavy ring, formation of precipitate

+++ strongly marked ring

++ marked ring

+ weakly marked ring

The results of the reaction were computed 5 and 20 minutes and 1 and 2 hours after storing the test tubes at room temperature.

Every experiment was conducted three times. The results, set out in Tables 1-3, recurred without change.

EXPERIMENTAL RESULTS

The results of the ring precipitation reaction with antiserum against the serum of an adult duck (Table 1) showed that the crystalline lens of the duck in all the studied stages of ontogenesis possesses a marked antigenic species specificity. The saline extracts of the crystalline lenses of the embryos in early stages of development (5, 8 and 11 days), gave a reaction at an attenuation of 1 : 200, and the extracts of the crystalline lenses of the embryos with 15 days incubation and of adult ducks did not give a positive reaction at this attenuation. This apparently points to reduction of the species specific antigenic properties of the crystalline lens in the process of development. In the control experiments with antigens from the brain and heart, such a reduction in the titer of reaction was not observed.

The conclusions of some authors [8, 13 et al.] regarding the absence in the crystalline lens of the eye of adult animals of an antigenic species specificity, are based, it seems to us, on incorrectly conducted experiments. The authors of previous works on this question arranged the serological reaction according to the pattern "crystalline lens antiserum with saline extracts of other organs, and the blood serum of the same animal species". In our experiments, too, according to this scheme, the results were negative. We attribute absence of a positive reaction to the phenomenon of a complete entrapment. In the conditions of these experiments we obtained compounds, in which the antibody-antigen ratio was significantly less than in compounds forming in the area of equivalence. Under these conditions, as is known, precipitates do not form. A different result is obtained if one takes a serum with a large antibody titer against the species specific antigens, and as antigen uses the crystalline lens, in which case a precipitate is formed.

Thus, the use in the reaction of a highly species-specific serum allows one to detect the antigenic species specificity of the crystalline lens.

The results of the experiments set out in Table 2 show that antigenic organism specificity was well marked in all the studied stages of development of the crystalline lens.

The organism specificity of the crystalline lens according to the degree of development is considerably raised. So, the antigen of the crystalline lens of 5-day old embryos reacted with the crystalline lens. The antiserum of the adult duck at an attenuation of 1 : 1000, the antigen of the crystalline lens of the 8-day embryos at an attenuation of 1 : 2500, and the antigen of the crystalline lens of 11-day embryos at an attenuation of 1 : 10,000, and finally, antigens of crystalline lenses of 15-day-old embryos and adult ducks – of 1:20,000.

TABLE 1

Results of Ring Precipitation Reaction Between Rabbit Serum Against Duck Serum and Antigens of the Organs of Duck Embryos at Different Stages of Development

Sera	Attenuation of the antigen	Antigens of the Embryonic Organs														
		5 days incubation			8 days incubation			11 days incubation			15 days incubation			Adult duck		
		Crystalline lens	Brain	Heart	Crystalline lens	Brain	Heart	Crystalline lens	Brain	Heart	Crystalline lens	Brain	Heart	Crystalline lens	Brain	Heart
Serum of Rabbit No. 568 against blood serum of duck	1 : 50	+++	+++	+++	+++	+++	++++	+++	+++	++++	+++	+++	++++	++	+++	++++
	1 : 100	++	++	++	++	+++	+++	++	+++	+++	++	++	+++	++	++	+++
	1 : 200	+	+	++	+	++	++	+	+	++	0	+	++	0	+	++
	1 : 300	0	0	0	0	0	+	0	0	+	0	0	+	0	0	++
	1 : 500	0	0	0	0	0	0	0	0	0			0			0
Normal blood serum of Rabbit No. 568	1 : 50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1 : 100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1 : 200	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

TABLE 2

Results of Ring Precipitation Reaction between Rabbit Serum Against Crystalline Lens of Duck and Antigens of Embryonic Organs at Different Stages of Development.

Sera	Attenuation of the antigen	Antigens of the Embryonic Organs														
		5 days incubation			8 days incubation			11 days incubation			15 days incubation			Adult duck		
		Crystalline lens	Brain	Heart	Crystalline lens	Brain	Heart	Crystalline lens	Brain	Heart	Crystalline lens	Brain	Heart	Crystalline lens	Brain	Heart
Serum of Rabbit No. 935 against crystalline lens of adult duck	1 : 50	++++	0	0	++++	0	0	++++	0	0	++++	0	0	++++	0	0
	1 : 100	++++	0	0	++++	0	0	++++	0	0	++++	0	0	++++	0	0
	1 : 300	++++	0	0	++++	0	0	++++	0	0	++++	0	0	++++	0	0
	1 : 500	++	0	0	+++	0	0	+++	0	0	++++	0	0	++++	0	0
	1 : 1 000	++	0	0	+++	0	0	+++	0	0	+++	0	0	+++	0	0
	1 : 2 500	0			++	0	0	++	0	0	+++	0	0	+++	0	0
	1 : 5 000				0			++	0	0	++	0	0	++	0	0
	1 : 10 000							+	0	0	++	0	0	++	0	0
	1 : 20 000							0			+	0	0	+	0	0
	1 : 40 000										0			0		
Normal serum of Rabbit No. 935	1 : 50	0			0			0			0			0		
	1 : 100	0			0			0			0			0		
	1 : 300	0			0			0			0			0		

TABLE 3

Results of Ring Precipitation Reaction Between Rabbit Serum Against Crystalline Lens of Duck and Antigens of Crystalline Lenses of Duck, Hen and Frog

Sera	Attenuation of antigen	Antigens of the Crystalline Lenses		
		Duck	Hen	Frog (Rana ridibunda)
Serum of Rabbit No. 935 against crystalline lens of duck	1 : 50	++++	++++	++++
	1 : 100	++++	++++	++++
	1 : 500	++++	+++	++
	1 : 1 000	+++	+++	++
	1 : 2 500	+++	++	+
	1 : 5 000	++	+	0
	1 : 10 000	++	+	0
	1 : 20 000	+	0	
	1 : 40 000	0		
Normal blood serum of Rabbit No. 935	1 : 50	0	0	0
	1 : 100	0	0	0
	1 : 500	0	0	0

It should be stressed that the saline extract of the crystalline lenses of the embryos after 15 days incubation gave a positive reaction at the same attenuation as the extract of the crystalline lens of the adult duck. The crystalline lens in this period of development acquired in terms of basic features the morphological structure peculiar to this organ in an adult state.

Thus, the results of these experiments allow one to assume the existence of a close relation between the morphological and antigenic changes, which take place in the process of development of the organism.

We believe that a similar mutual relationship must be observed in phylogenesis, i. e., the more closely related the organs in the morphological sense, the greater must be their antigenic resemblance. In order to verify this hypothesis a series of experiments was conducted, the results of which are set out in Table 3.

As can be seen from Table 3, the antigenic similarity of the crystalline lenses of the duck and hen are considerably greater than the duck and frog. The morphological structure of the crystalline lenses of the duck and hen are very similar, while in terms of morphological structure, the crystalline lens of a frog considerably differs from that of the duck. The results of the experiments in this series, in our judgement, on a first calculation point to a relationship of morphologic and antigenic changes, which take place in phylogenesis.

In general, the results of our experiments showed the following:

The crystalline lenses in all the stages of ontogenesis studied possessed a marked antigenic species specificity. The antigenic organism specificity of the crystalline lenses was clearly expressed in all the stages of development studied. The organism specificity increased in line with the degree of development. There was seen a parallel change in the morphologic and antigenic properties, both in ontogenesis and phylogenesis. The findings obtained by means of the ring precipation reaction fully concur with the results which were obtained with the aid of the anaphylaxis reaction, and are outlined in our previous communications [2, 3].

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