A COMPARATIVE STUDY OF THE ANTIGENIC PROPERTIES OF THE CRYSTALLINE LENS IN VERTEBRATES AND INVERTEBRATES

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Uhlenhuth [22] demonstrated the similar antigenic properties of the crystalline lens in various vertebrate animals. According to this worker, the crystalline lenses of mammals, birds and amphibia contain identical proteins (antigens), which are also present in traces in the crystalline lenses of fishes. He considered that this phenomenon was the result of the identical function of the crystalline lens in vertebrates. These findings have been confirmed by several workers [1, 5, 7, 10-16, 20 and others].

Wollman, Gonzalez and Ducrest [24] used the precipitation reaction to study the antigenic properties of the crystalline lenses of the Cephalopoda and of vertebrates. These workers reported that the antilens serum of the octopus (Octopus vulgaris) did not react with antigens of the crystalline lenses of fishes and mammals, and that antilens sera of fishes and mammals did not react with antigens of the crystalline lens of the octopus. Nevertheless Tutui [21] showed that positive precipitation and complement fixation reactions were not observed only when the antigens used in the tests or in preparing the antisera consisted of extracts of the crystalline lenses of very unrelated species of animals (for example ox and cuttle-fish). If the difference between the species was less, the reaction was stronger and clearer.

Thus the problem of the similarity or difference in antigenic properties of the crystalline lenses of the vertebrates and invertebrates has not yet been finally solved, and accordingly we decided to carry out an investigation ourselves.

EXPERIMENTAL METHOD

We studied the antigenic properties of the crystalline lenses of birds (chick, goose), mammals (guinea pig), reptiles (grass snake, Natrix natrix), amphibia (frog, Rana ridibunda), fishes (cod, Godus callarias) and cephalopod molluscs (calamary, Ommatostrephes sagittatus).

In the first series of experiments we studied the antigenic properties of the crystalline lenses of vertebrates by means of the ring precipitation test [5]. Antisera were obtained in rabbits against the water-soluble antigens of the crystalline lens of the chick, by the method suggested by P. N. Kosyakov [6]. The antisera reacted with antigens of the lens of the chick in dilutions of the latter of 1:40,000. These antisera were highly specific and did not react with saline extracts of other organs nor with the blood serum of the chick.

In the second series of experiments we studied the antigens of the crystalline lenses of vertebrates by means of the precipitation test in agar in capillary tubes.

In the third series of experiments we made a comparative study of the antigenic properties of the crystalline lenses of vertebrate birds and fishes and invertebrates (cephalopod molluscs), using the anaphylaxis reaction in guinea pigs [4]. The guinea pigs were sensitized subcutaneously with suspensions of formalized (in 8% formalin)

TABLE 1

Results of the Ring Precipitation Test between Rabbit Antiserum (No. 3261) to the Crystalline Lens of the Chick and Antigens of the Lenses of Various Species of Animals

Antigens of the lens of	Dilution of antigens							
	1:500	1:1 000	1:2 500	1:5 000	1:10 000	1:20 000	1:40 000	1:80 000
Chick	++++	+++	+++	+++	++	+	+	—
Goose	++++	+++	+++	++	+	+	—	—
Guinea pig	+++	+++	++	++	+	+	—	—
Grass snake	+++	+++	++	++	+	+	—	—
Frog	+++	+++	++	++	+	—		
Cod	+++	++	+	+	—			

Note. + + + + dense ring, formation of a precipitate; + + + well marked ring; + + marked ring; + slight ring; - no ring.

TABLE 2

Results of the Precipitation Reaction in Agar between Rabbit Antiserum (No. 3261) to the Crystalline Lens of the Chick and Antigens of the Lenses of Various Species of Animals

Antigens of the lens of	Number of rings of precipitate			
	antiserum to the lens			Normal rabbit serum (No. 3261)
	dense rings	slight rings	total number of rings	
Chick	4	3	7	0
Goose	4	1	5	0
Guinea pig	2	2	4	0
Grass snake	3	0	3	0
Frog	1	1	2	0
Cod	0	Very slight clouding of the agar	0	0
Chick's liver	0	0	0	
Chick's heart	0	0	0	
Chick's brain	0	0	0	

lens tissue of the chick, cod and calamary. The lens tissue was carefully washed free from formalin in tap water. On the 21st day after sensitization, all the guinea pigs were injected intravenously with saline extracts (1:10) of nonformalized lens tissue of the chick or cod. These extracts were also injected intravenously into 4 nonsensitized guinea pigs (technical control). As a further control we used extracts (1:10) of the liver of the cod and chick, which were injected into guinea pigs sensitized by antigens of lens tissue of the chick, cod and calamary.

EXPERIMENTAL RESULTS

It was shown by means of the ring precipitation test with antisera to antigens of the crystalline lens of the chick (Table 1) that the lenses of all the vertebrates studied (chick, goose, guinea pig, grass snake, frog and cod) possess similar antigenic properties. Saline extracts of the lens tissue of the chick reacted in a dilution of 1:40,000,

TABLE 3

Anaphylaxis Reaction in Guinea Pigs Sensitized to Crystalline Lens Tissue of the Chick, Cod and Calamary, in Response to the Injection of an Extract of the Crystalline Lens Tissue of the Cod or Chick

	Sensitization. Antigen-suspension of crystalline lens tissue (does 5 mg)	Assaulting injection. Antigen-extract of crystalline lens tissue of the cod (dose 500 mg/reaction)	Sensitization. Antigen-suspension of crystalline lens tissue (dose 5 mg)	Assaulting injection. Antigen-extract of crystalline lens tissue of the chick (dose 500 mg/reaction
	Chick	+	Chick	++++
	»	++	»	+++
	»	+	»	+++
	Cod	+++	Cod	+
	»	++	»	+
	»	+++	»	+
	Calamary	++	Calamary	—
	»	+	»	+
	»	++	»	—
		Injection of extract of cod liver tissue (dose 500 g/reaction)		Injection of extract of chick's liver tissue (dose 500 mg/reaction)
Control	Chick	—	Chick	—
	»	—	»	—
	Cod	—	Cod	—
	»	—	»	—
	Calamary	—	Calamary	—
	»	—	»	—
		Injection of extract of crystalline lens tissue of the cod (dose 500 mg/reaction)		Injection of extract of crystalline lens tissue of the chick (dose 500 mg/reaction)
	No sensitization produced (technical control)		No sensitization produced (technical control)	—
	The same	—	The same	—

Note. + tremor, running nose and eyes, shaggy fur, dyspnea, slight fall of temperature; + + the same signs more strongly expressed; the guinea pig often sneezes; + + + the same signs more strongly expressed; convulsions, cough, animal lies on its side but survives; + + + + all signs expressed very strongly, animal dies; - no signs of anaphylactic shock.

and extracts of the lens tissue of the goose, guinea pig and grass snake — in a dilution of 1 : 20,000, frog — 1:10,000 and cod — 1:5000. The results of these experiments show that the further apart the animals are in their position in the system, the less the antigenic similarity of their crystalline lenses.

The results obtained by the ring precipitation test do not show sufficiently clearly, however, the identity of or difference between the antigenic properties of the crystalline lenses of different animals. We thought that this was best demonstrated by the precipitation reaction in agar. As a result of a large number of experiments it was shown (Table 2) that during the interaction of antiserum to the antigens of the crystalline lens of the chick with homologous antigens, 7 rings of precipitate are formed (4 dense and 3 slight), during the interaction with antigens of the lens of the goose – 5 rings (4 dense and 1 slight), with antigens of the lens of the guinea pig – 4 rings (2 dense and 2 slight), with antigens of the lens of the grass snake – 3 dense rings, with antigens of the frog's lens – 2 rings (1 dense and 1 slight) and with antigens of the lens of the cod – 1 very slight clouding of the agar.

If, however, as antigens were used extracts from other organs of the chick (heart, liver, brain), no rings of precipitate were formed in the agar. From the results obtained it can be assumed that there are two different types of organ-specific antigens: organ-specific antigens common to the same organ in different species of animals, and organ-specific antigens which are characteristic for that particular organ in the particular species of animal alone. It may be thought that the presence of common organ-specific antigens in the organs of different species of animals is associated with the common function of these organs and with the family relationships between the different species of animals from the evolutionary aspect, whereas the presence of antigens which are both organ-specific and species-specific is associated with the peculiarities of the function and structure of the organ in the given species of animal.

The results of the second series of experiments show that the more closely related the animals are to each other, the higher the content of common organ-specific antigens in their crystalline lenses and also that the lenses, and also that the lens of the animals of the group Amniota (mammals, birds and reptiles) differ markedly from those of animals of the group Anamnia (amphibia and fishes). Of interest in this connection are the results of experiments with antigens from the crystalline lens of a fish (cod), since during interaction of these antigens with antisera to antigens from the lens of the chick in an agar gel, only slight clouding is produced. This shows that the crystalline lenses of fishes and birds evidently contain no common antigens, but only the common specific determinants of the group, which are responsible for the similarity in the antigenic properties of the crystalline lenses of these two far-removed classes of animals. In support of this are the results obtained by electrophoresis [23]. It may be thought that the presence of common group determinants in the protein molecules of the lenses of very distantly related animals (fishes and birds) is associated with the identical function of the crystalline lens – the transmission of light.

We were confronted with the problem of whether the crystalline lenses of vertebrates and invertabrates have any common antigenic properties. The eyes of the Cephalopoda are of greatest interest, for in their structure and function they are very similar to the eyes of vertebrates [2, 17, 18]. However the eyes of the cephalopod molluscs – and in particular the crystalline lens – have a completely different origin from those of vertebrates [3, 9, 19 and others], i.e. the eyes of the cephalopod molluscs and the vertebrates are analogous organs and afford a striking example of convergent homology. For this reason the comparative study of the antigenic properties of the crystalline lenses of the cephalopod molluscs and the vertebrates is of great interest.

The results obtained by means of the anaphylaxis reaction showed (Table 3) that all the guinea pigs sensitized to antigens of the crystalline lens of the chick, cod and calamary, responded to injection of saline extract of lens tissue of the cod by marked signs of anaphylactic shock. However, in response to injection of saline extract from lens tissue of the chick into guinea pigs sensitized with antigens from the crystalline lens of the calamary, we observed a feebly developed anaphylactic shock in one animal only and in the other two guinea pigs no signs of anaphylactic shock could be seen.

The absence of anaphylactic shock in the two guinea pigs is evidently due to the fact that the antigens of the calamary used for sensitization have very few determinant groups similar to those of the antigens used for the assaulting injection (saline extract of lens tissue of the chick). At the same time, injection of a saline extract of crystalline lens tissue of the chick into guinea pigs sensitized with antigens of the lens of the chick and cod, signs of anaphylactic shock were observed. After injection of saline extracts of the liver tissue of the cod and chick into guinea pigs, sensitized with antigens of the crystalline lens of the chick, cod and calamary, we observed no signs of anaphylactic shock. Injection of saline extracts of the crystalline lens tissue of the cod into nonsensitized guinea pigs also gave no signs of anaphylactic shock.

Thus the results obtained by the anaphylaxis reaction show that there is homology of the antigenic properties of the crystalline lenses of the cephalopod molluscs and the vertebrates and moreover that this homology is strong between the crystalline lens antigens of the cod and calamary, and almost imperceptoble between the lens antigens of very distantly related species — the chick and calamary. Hence it follows that the performance of identical functions by organs leads not only to homology of their structure but also to homology of their antigenic properties, in spite of their different origin. These findings are in agreement with the experimental results of I. I. Titova [8], who demonstrated the homology of the antigenic properties of the crystalline lens tissue of the triton, which is derived in embryogenesis from the ectoderm and the crystalline lens formed the iris during regeneration.

SUMMARY

The results of our experiments using the precipitation test in agar showed that the presence of common antigens in the crystalline lenses of various animals is associated not only with convergence, but also to a large degree, evidently, with the family relationships between the various species of animals. Hence, in the comparative study of the antigenic properties of organs in different animals it is essential to take into consideration the degree of relationship between the animals and the possibility of convergent homology of the organs resulting from performance of the same function.

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