

THE STUDY OF THE ANTIGENIC PROPERTIES OF TISSUES AND ORGANS OF ANIMALS IN ONTOGENESIS

COMMUNICATION V. THE STAGE-SPECIFIC ANTIGENS OF THE CRYSTALLINE LENS

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In previous communications we discussed the question of the appearance of species and organ antigenic specificity of the crystalline lens of the duck and of the heart of the chick [4,5,6,7]. Undoubtedly the complex structural changes undergone by organs in the process of development are closely related to metabolic changes and consequently, to changes of the protein biosynthesis type. Changes of metabolism and of protein biosynthesis in their turn must lead to changes in the antigenic properties of certain organs. From this starting point we set out to answer the problem of whether there are specific antigens for each stage of development of the crystalline lens.

There are reports in the literature suggesting that in the embryonic period of development, the serum [2,11,17,19], erythrocytes [12,14,15,16,20], muscular tissue [9,10,18] and other organs [1] of man and various animals contain proteins (antigens) which differ from the corresponding proteins (antigens) of the adult.

We were able to find only one work in which the writers demonstrated the presence in the crystalline lens of the chick of antigens specific for the definite stages of development. V. Burke, N. Sullivan, H. Petersen and R. Weed [13] report that antigens of the crystalline lens of chick embryos 96 hours after incubation react with anti-crystalline lens serum of embryos 160 hours after incubation but do not react with such serum 300 hours after incubation, whereas, antigens of the crystalline lens of embryos 120 hours after incubation react with anti-lens serum of embryos 300 hours after incubation but not with anti-lens serum of adult chicks. Unfortunately the writers did not make a detailed study of the morphogenesis of the crystalline lens, and therefore on the basis of their data, it is difficult to connect the presence of certain antigens at definite stages of development with any of the morphological features which are characteristic of the crystalline lens at these stages. As we know, the crystalline lenses of 300 hour chick embryos and those of mature chicks are very similar in structure. The question of why the crystalline lenses of 120 hour embryos reacted with anti-lens sera of 300 hour embryos, but not with anti-lens sera of mature chicks, remains unsolved.

The present investigation was undertaken with the object of further clarification of this problem.

EXPERIMENTAL METHODS

In this work we used the reaction of anaphylaxis with desensitization [3]. The experiment was set up as follows. Guinea pigs (15 in number) were sensitized with suspensions of crystalline lens tissue of 90, 130, 192, 264 and 360 hour duck embryos. On the 21st day after the sensitizing injections the animals were desensitized to species and organ specific antigens (2 intraperitoneal injections of extract from the crystalline lens of an adult duck). Two hours after the second desensitizing injection a test of the completeness of de-

sensitization was carried out. Two hours after this, the animals received intravenously a determining injection of antigen. For the determining injections we used the same antigens which were used for sensitizing the animals.

EXPERIMENTAL RESULTS

The experimental results are shown in Table 1. As can be seen from this Table, in all the sensitized animals in response to the injection of extract * from crystalline lens tissue of an adult duck, there was observed a marked positive reaction; some of these animals even died from anaphylactic shock. On testing the completeness of desensitization, no positive anaphylactic reaction was detected. The animals which were sensitized to the lens of 90 and 130 hour embryos gave a positive reaction, assessed as "++", in response to the determining injections. Guinea pigs which were sensitized to suspensions of lens tissue of 192, 264 and 360 hour duck embryos did not give a positive reaction in response to the determining injection of antigen.

Thus the experimental results given in Table 1 show that in the crystalline lens tissue of 90 and 130 hour embryos besides the species and organ specific antigens which are common to the lens of the adult duck, there are also found antigens, specific for these stages of development.

However, giving our attention to the results of previous experiments [4] in which it was shown that the antigenic species specificity of the crystalline lens was very prominent in early stages of its development, we became doubtful of the completeness of desensitization to species specific antigen of the guinea pigs which had been sensitized to the lenses of 90 and 130 hour embryos. To settle this point we made the following experiment.

Guinea pigs (9 in number) were sensitized to suspensions of crystalline lens tissue of 130, 192 and 360 hour embryos. On the 21st day after the sensitizing injections, all the animals were desensitized to species and organ specific antigens (two subcutaneous injections of extract of lens tissue from adult ducks the day before carrying out the reaction). On the morning of the day the reaction was to be performed, tests were made of the completeness of desensitization to organ specific antigen (intravenous injection of extract of lens tissue of an adult duck). If the guinea pigs did not show a positive reaction, then 2 hours later tests of completeness of desensitization to species specific antigen were made by the intravenous injection of adult duck serum. Two hours after these tests the animals received intravenously the determining injection of antigen. For this purpose we used the same antigens which had been used to sensitize the animals.

The result of this experiment are shown in Table 2, from which it can be seen that the test of completeness of desensitization to species specific antigen did not give a positive reaction.

Guinea pigs sensitized to crystalline lens tissue of 130 hour embryos gave a positive reaction, classed as "+", in response to the determining dose of antigen. At the same time, the animals sensitized to lens tissue of 192 and 360 hour embryos showed no positive reaction in response to the determining injection of antigen. Two unsensitized guinea pigs were injected intravenously with extract of lens tissue of 130 hour duck embryos (technical control), and these did not show a positive reaction.

The results of this experiment confirm the findings of the previous experiment on the presence in tissue of the crystalline lens of embryos of an early stage of development (90 and 130 hours incubation) of antigens specific for these stages. We called these antigens "stage specific" [8].

The morphological investigation which was performed showed that at this period of development (90-130 hours) intensive growth of the primary lens fibers was observed, determining the high rate of growth of the entire crystalline lens (see Figure) **. Estimation of ribonucleic acid (RNA) by Brashe's method showed also

* The preparation of the extract was described previously by us [4].

** In constructing this graph we used the results of calculations of the volume of the lens in embryos of various stages of incubation (3-4 embryos on each day). The volume of the lens was determined in histological specimens by the formula for the volume of a revolving ellipsoid.

Anaphylaxis Reaction in Guinea Pigs Sensitized with Suspensions of Duck Embryo Crystalline Lens Tissue in Response to Injection of Lens Tissue Extract from Fully Grown Ducks and Embryos

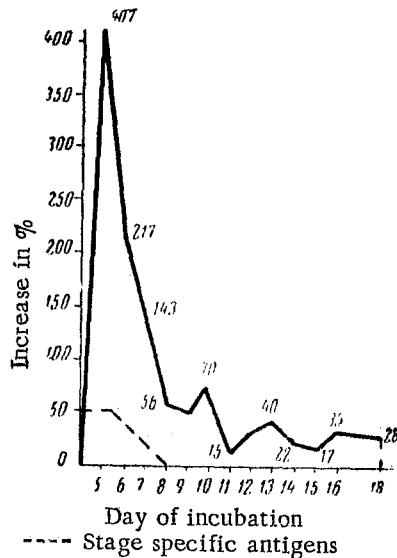
* Desensitization was performed fractionally: 2 injections of 250 mg after 6 hours (some animals died from anaphylactic shock from the first injection).
2* 3* 4* In view of the great difficulty of extracting and determining the exact weight of the crystalline lenses in 90 hour embryos, in the preparation of 200 mg of extract we took not 20 mg of tissue as usually, but 300 lenses.
Note. + + + death of the animal from anaphylactic shock; + + + severe shock but the animal survived; + + well marked shock; + slight shock; + clear signs of shock absent; — total absence of any signs of shock.

TABLE 1 (Continued)

Anaphylaxis Reaction in Guinea Pigs Sensitized with Suspensions of Duck Embryo Crystalline Lens Tissue in Response to Injection of Lens Tissue Extract from Fully Grown Ducks and Embryos

Sensitization (subcutaneously)		Desensitization* (intraperitoneally)			Test complete of desensit. (intravenously)		Determining injection (intravenously)	
Antigen	Dose	Antigen	Dose (mg)	Reaction	Antigen	Dose (mg)	Antigen	Dose (mg)
Suspension of crystalline lens tissue of 264 hr. duck embryos	1.6 ml	Extract lens tissue fully grown ducks	500	++++	Extract lens tissue fully grown ducks	400	Extract lens tissue 264 hr. duck embryos	200
The same	1.6 »	The same	500	+++				
» » » » » » » » » »	1.6 »	» » » » » » » » » »	500	++	The same	400	The same	200
Suspension of crystalline lens tissue of 360 hr. duck embryos	1.6 »	» » » » » » » » » »	500	++	» » » » » » » » » »	400	Extract lens tissue 360 hr. duck embryos	200
The same	1.6 »	» » » » » » » » » »	500	++	» » » » » » » » » »	400	The same	200
» » » » » » » » » »	1.6 »	» » » » » » » » » »	500	++	» » » » » » » » » »	400	» » » » » » » » » »	200
Suspension of crystalline lens tissue of fully grown ducks	1.6 »	» » » » » » » » » »	500	++	» » » » » » » » » »	400	Extract lens tissue fully grown ducks	200
The same	1.6 »	» » » » » » » » » »	500	++	» » » » » » » » » »	400	The same	200
» » » » » » » » » »	1.6 »	» » » » » » » » » »	500	++	» » » » » » » » » »	400	» » » » » » » » » »	200

that in the period of intensive growth of the lens its RNA content increased in concentration. It is interesting that stage specific antigens are found in lens tissue of embryos of just this period of incubation. On the 8th day of incubation, when stage specific antigens can no longer be detected, the process of formation of secondary lens fibers begins from the lens epithelium. The primary lens fibers at this time begin to undergo conversion into the "nucleus" of the lens. The RNA content of the lens tissue, and also its rate of growth are sharply reduced on the 8th day of incubation. It may be that the presence of stage specific antigens in the lens tissue of 90 and 130 hour embryos is connected with the above mentioned morphological features of the developing lens in this period of embryogenesis.



Rate of growth of the crystalline lens of the duck with days of incubation.

The findings reported in this article do not confirm the results of the experiments of Burke and his co-workers [13] mentioned above. Our experimental results showed that stage specific antigens, by contrast with the findings of Burke and his co-workers, are present in lens tissue only during early stages of development. Thus the findings of the present work show that in the developing lens tissue of the duck in early stages of embryogenesis (90 - 130 hours) antigens are present which are specific only for these stages of development - stage specific antigens. At this period the formation and intensive growth of the primary lens fibers occurs and an increased concentration of RNA is characteristic of the lens tissue.

SUMMARY

The antigenic properties of the tissue of developing crystalline lens of ducks was studied with the aid of anaphylactic reaction with desensitization.

Morphogenesis, the rate of growth of crystalline lens and content of ribonucleic acid (RNA) in it were investigated simultaneously. It was demonstrated that dur-

ing the early stages of embryogenesis (90-130 hours) the antigens are present in the tissue of developing crystalline lens, which are specific only for the definite stage of development (stage-specific antigens). There is formation and intensive growth of the primary fibers of the crystalline lens and increased concentration of the RNA during this period.

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* Original Russian pagination. See C. B. Translation.

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