

# EXPERIMENTAL BIOLOGY

## A STUDY OF THE ANTIGENIC PROPERTIES OF THE CRYSTALLINE LENS IN WOLFFIAN REGENERATION

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One aspect of the study of the rules regulating the formative processes is the investigation of the minute changes in the protein composition of the developing tissues, which finally result, evidently, in a change in the morphological structure of these tissues. Since the change in tissue protein composition is attended by a change in the antigenic properties of the tissues, studying these properties can definitely help solve the problem regarding the mechanism of the formative processes.

It has recently been established that the tissues of the various animal and human organs particularly contain antigens specific to the given organ alone (organo-specific antigens). It has also been established that the various organo-specific antigens appear at definitely fixed stages of embryonal development [3, 4, 7].

The most generally accepted point of view is that the presence of an organo-specific antigen is connected with a definite function being fulfilled by the given organ [5, 6, 8].

However, in 1956, B. V. Konyukhov published a work presenting data showing that the organo-specific antigen of the crystalline lens could be found in the placode stage, i.e., at a period when the actual function of the crystalline lens does not exist.

There is a question which must be specifically settled in connection with this: do organo-specific antigenic properties depend on the fact that the given organ is carrying out a definite function, or are these properties dependent on the origin of the given tissue from a definite tissue source?

The purpose of our work was to solve this problem.

We used the phenomenon of so-called wolffian regeneration of the crystalline lens in our work.

When the crystalline lens is removed from a triton's eye, the triton is known to form a new crystalline lens, not in the usual way - from the ectodermal epithelium, but from the upper edge of the iris, i.e., from tissue derived from the neural tube. Therefore the crystalline lens formed by wolffian regeneration performs the same function as an ordinary crystalline lens, but has a different origin.

It was important to establish whether the tissue formed in wolffian regeneration of the crystalline lens would have the same antigenic properties as the tissue of an ordinary crystalline lens or whether these properties would differ according to the difference of tissue origin.

The solution of the question has not been described in the literature.

## EXPERIMENTAL METHODS

The crystalline lens was removed from the right eye of tritons (*Tr. taeniatus*) under ether anesthesia. The tritons were fastened down in a small paraffin bath by fine needles in such a way that the right eye was uppermost. The cornea was carefully slit with a lanceolate knife and a glass rod, and the crystalline lens removed with finely pointed pincers.

The animals which had been operated upon were kept for 5 and for 15 days. After this, their iris tissues were removed and ground with a few drops of a physiological solution of NaCl. The resulting paste was used in the subsequent experiments.

To study the antigenic properties of the tissues forming from wolffian regeneration, we used the anaphylactic reaction, the use of which in determining the antigenic properties of embryonic tissues was demonstrated earlier by B. V. Konyukhov and R. F. Averkina [1, 2, 3].

Guinea pigs were sensitized subcutaneously with an emulsion from the tissue of 20 irises, isolated 5 and 15 days after the removal of the crystalline lens. Guinea pigs sensitized by an emulsion from the same quantity of iris tissues, isolated from the same species of animal in which the eyes had not been operated upon, were used as the control.

On the 30th day after sensitization, all the animals were injected intravenously with 60 mg of a water-salt extract from the crystalline lens tissue of a frog (*Rana ridibunda*).

We chose the crystalline lens of the frog, rather than of the triton, in order to avoid the possibility of a reaction from species-specific antigens.

The extract for the reacting injection was prepared as follows. The crystalline lenses were carefully ground in a porcelain mortar, and 9 volumes of a physiological solution of NaCl were added. The resulting emulsion was shaken for 20 minutes and then centrifuged for 20 minutes at a speed of 2,000 revolutions per minute.

The degree to which the anaphylactic reaction was expressed was determined by the usual method [2].

## EXPERIMENTAL RESULTS

The results of the experiments described are given in the table.

Anaphylactic Reaction in Guinea Pigs, Sensitized by Iris Tissues, in Response to Reacting Injection of the Crystalline Lens Extract From a Frog

| No. of guinea pig | Sensitization  |               | Reaction injection |              | Reaction |
|-------------------|--|---------------|--------------------|--------------|----------|
|                   | Antigen  | Dose (irises) | Antigen            | Dose (in mg) |          |
| 1067              | Iris on the 5th day after removal of the crystalline lens  | 20            | Crystalline lens   | 60           | —        |
| 1034              | The same   | 20            | The same           | 60           | —        |
| 1363              | The same   | 20            | » »                | 60           | —        |
| 1926              | Iris on the 15th day after removal of the crystalline lens | 20            | » »                | 60           | +        |
| 1943              | The same   | 20            | » »                | 60           | +++      |
| 1906              | The same   | 20            | » »                | 60           | +        |
| 1312              | Iris from eyes not operated upon                           | 20            | » »                | 60           | —        |
| 1313              | The same   | 20            | » »                | 60           | —        |
| 1792              | The same   | 20            | » »                | 60           | —        |

As the table shows, the reaction to the reacting injection of the antigen from the tissues of the frog crystalline lens tissue was negative in all of the guinea pigs which had been sensitized by the emulsion from the tissues of irises taken from the tritons on the 5th day after removal of the crystalline lens. All the guinea pigs sensitized by the emulsion from the iris tissue taken from the tritons on the 15th day after removal of the crystalline lens, however, gave a positive anaphylactic reaction in response to the reacting injection of the antigen.

The reaction was negative in the control animals.

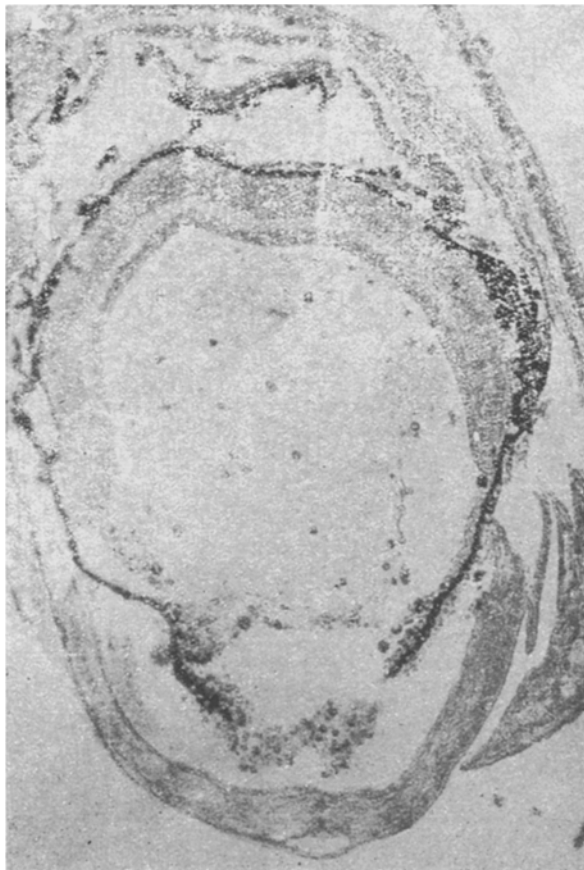


Figure 1. Early stage of crystalline lens formation in triton. Edge of iris breaking up, beginning of iris depigmentation. The crystalline lens antigen was not observed at this stage (5th day of experiment).

Therefore, the organo-specific crystalline lens antigen was discovered in the iris tissue on the 15th day after the removal of the crystalline lens. This antigen was not found on the 5th day.

On the 5th day after the crystalline lens had been removed, the crystalline lens formation process had just begun. At this stage, the edge of the iris was breaking up and beginning to become depigmented (Fig. 1). By the 15th day, there was already a well-formed, although not yet completely separated, crystalline lens on the upper edge of the iris (Fig. 2). The crystalline lens which had formed from the iris was exactly like the ordinary crystalline lens in its histological structure.

Therefore, the specific, crystalline lens antigen could be observed in a crystalline lens, when sufficiently formed, which was derived from the iris. Although, due to the reasons given earlier, only one organ was used in the work, i.e., the crystalline lens, our data allow the proposition that organs fulfilling the same function and having the same histological structure possess similar antigenic properties, although their origin may not be the same.

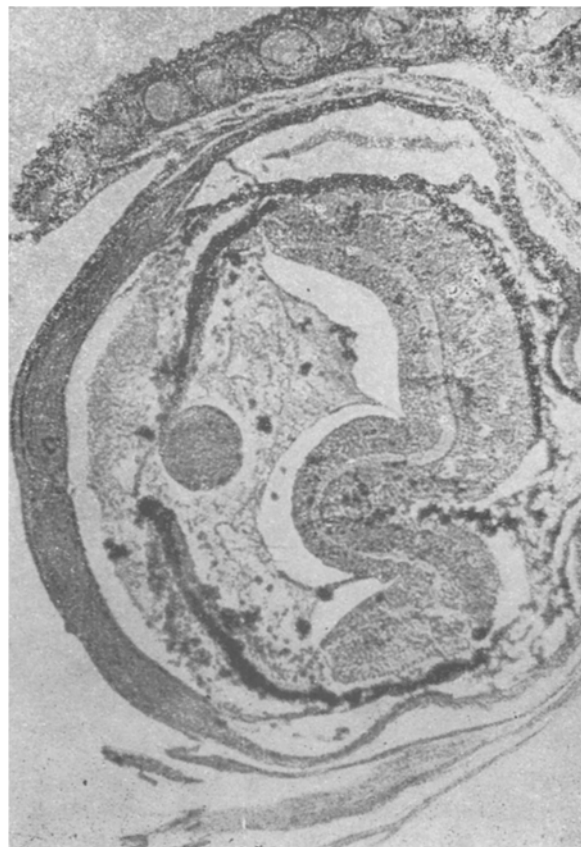


Figure 2. Crystalline lens which has formed from the iris. The antigens specific to the crystalline lens were clearly evident at this stage (15th day of experiment).

Of course, the fact that the same antigens are present in the ordinary crystalline lens and in the regenerated crystalline lens does not exclude the possibility that there are also different antigens in these tissues, which owe their presence to the difference in the origin of the tissues. However, we are now conducting the special study which is needed to solve this problem.

#### SUMMARY

Experiments on tritons have shown that on the 15th day of wolffian regeneration of the crystalline lens normally extirpated from the iris, an antigen specific for the crystalline lens appears in that tissue. No antigen could be detected at earlier stages of regeneration (on the fifth day). It is supposed that organs of the same function and histologic structure, no matter of what origin they are, have identical antigenic properties.

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