# THE ORGAN SPECIFIC PROPERTIES AND ANTIGENIC POWER IN THE HOMOLOGOUS SPECIES OF ALPHA CRYSTALLIN*

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Uhlenhuth,[1] in 1903, announced his discovery of the organ specificity of lens protein. Briefly summarized, organ specificity means that the chemical structure of an organ or tissue is the same in all species, and differs from the general tissue proteins so markedly that the organ specific substance is capable of acting as a foreign protein. Specifically, when lens extracts are injected into rabbits or other species, the injected animal responds by producing precipitins and complement-fixing bodies which react in the test tube with lens extracts from all vertebrate species. In our studies lens extracts have been found to fulfil the above specification and to be organ specific, except in the particular that whole lens extract did not act as an antigen in the homologous animal. It is the purpose of this paper, therefore, to describe our studies of this exception, as well as our experiments with whole lens and its fractions when injected into the homologous species.

Hektoen and Schulhof[2] found, as we have, that rabbits injected with beef or pig lens promptly develop precipitins

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which react in the test tube with lens extracts of other animals. They also found, however, that if rabbit lens extracts are injected into rabbits, there is no precipitin formation. To produce lens antibodies in the homologous species these investigators gave the rabbits a preliminary immunization with beef lens extract. The rabbits were then allowed to rest until all precipitins had disappeared from the blood stream, and were then injected with rabbit lens extract. Precipitins for lens extract from any species again appeared in the blood stream. This experiment is open to the criticism that the appearance of precipitins after the injection of the homologous lens extract may be due to a simple mobilization of previously formed and fixed antibodies, the homologous lens protein acting as a non-specific stimulus. Such a phenomenon has previously been described as the anamnestic reaction, the most common example of which is the rise in typhoid agglutinin titre following the injection of pyrogenetic substances, such as nucleic acid, or following an actual injection with some other group of organism.

In our preliminary experiments we were unable to confirm this experiment of Hektoen and Schulhof.[2] In any case the devious route by which Hektoen and Schulhof obtained such antibodies suggests that whole lens extract does not fulfil all the requirements of a true organ specific substance, because it is incapable of readily producing antibodies in the homologous species. This problem is of practical as well as academic importance, because it involves the whole question of auto-immunization and sensitization to lens and the associated picture of endophthalmitis phaco-anaphylactica. If, for example, it is impossible to immunize or sensitize a given species to its own lens substance, then the anaphylactic theory of the above disease is open to question.

In previous papers [3, 4, 5] we have reported upon the separation and purification of Alpha, Beta, and Gamma crystallins

present in lens extracts. During the course of these experiments, and in the associated skin testing of humans, it became obvious that Alpha crystallin was the most reactive fraction antigenically. When preliminary investigation had shown that rabbits could not be immunized to whole rabbit lens alone or by the Hektoen-Schulhof method, or that rabbits that were repeatedly needled produced no antibodies, we investigated the action of the individual purified crystallin in the homologous species.

The following experiments, the results of which are shown in Table I, have been performed over a period of four years. In these experiments rabbits, swine, and calves have been injected with homologous whole lens extract, Alpha crystallin, and a mixture of the Beta-Gamma crystallins.

The rabbit, swine, and beef lens extracts were prepared, and the separation of the fractions was done, in the manner previously described. The animals were all bled preceding the first injections, and these serums, negative to all antigens, were used as controls in the later titrations. The animals were injected intravenously twice a week with the various preparations. Test bleedings were done at intervals and the injections were discontinued when such serums showed definite antibodies or when a seemingly adequate number of injections gave entirely negative results. By "a seemingly adequate number of injections" we mean that the animals received at least the same number of injections and the same amount of protein per body weight as did the positive animals in the same series. In most of the negative animals the injections were continued for at least two weeks after animals in the same series had become positive. The calves were not injected with the Beta-Gamma complex, for the reason that this experiment had previously given negative results in both rabbits and swine, and the difficulties incident to the use of calves for experiments made it necessary to use as few animals as possible.

Table I contains, first, a summary of the previously published work—the results that follow the injection of heterologous whole lens and the purified crystallins. Secondly, it gives the results which follow the injection into three species—rabbits, swine, and calves—of homologous whole lens and the homologous purified crystallins. Thus heterologous whole lens produces antibodies which react with all whole lens antigens and the various crystallins. The heterologous crystallins produce antibodies which react with the specific crystallins and with whole lens antigens. Homologous whole lens, however, produces no detectable antibodies of any kind, and the homologous Beta-Gamma complex likewise appears inert. Homologous Alpha crystallin, however, produces antibodies specific for Alpha crystallin from all species. These antibodies also react with whole lens extract.

These results suggest that:

1. Alpha crystallin is a true organ specific substance, is the same in all species, is present in all whole lens extracts, and is not a product of chemical manipulation.

2. As a complex* Beta and Gamma crystallins are inert in the homologous species.

3. When combined with Alpha crystallin in whole lens extract, Beta and Gamma crystallins so color the antigenic mosaic that in the homologous species the antigenic properties of Alpha crystallin are inhibited.

These results suggest in a measure why, in the great majority of individuals, escape of lens substance through the traumatically or surgically ruptured capsule is not followed by the development of antibodies of an immune or allergic type. In such individuals in whom sensitivity is pre-existent or develops subsequent to capsule rupture, the explanation is not clear. Theoretically, a disappearance of Beta-Gamma

* In the above summary of results Beta and Gamma crystallins have been considered as acting alike. Results which are not yet on an adequately controlled basis suggest that Beta crystallin may be like Alpha crystallin, and that Gamma is the inhibiting or protective substance.

TABLE I

| Antigens | Serums of | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rabbits | | | Rabbits | | | Swine | | | Calves | | |
| | Injected with Beef Lens | | | Injected with Rabbit Lens | | | Injected with Swine Lens | | | Injected with Beef Lens | | |
| | Whole | Alpha | Beta and Gamma | Whole | Alpha | Beta aud Gamma | Whole | Alpha | Beta and Gamma | Whole | Alpha | Beta and Gamma |
| Whole Lens: Rabbit, Swine, Beef | + | + | + | 0 | + | 0 | 0 | + | 0 | 0 | + | Not done |
| Alpha Crystallin: Rabbit, Swine, Beef | + | + | 0 | 0 | + | 0 | 0 | + | 0 | 0 | + | Not done |
| Beta Crystallin: Rabbit, Swine, Beef | + | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | Not done |
| Gamma Crystallin: Rabbit, Swine, Beef | + | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | Not done |

crystallin from the lens substance may allow auto-immunization, or the individuals displaying such sensitivity may vary from the normal and have a different immunity mechanism. However, neither of these hypotheses is tenable without further investigation.

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# A REPORT OF AN EXPERIMENTAL STUDY OF THE REPARATIVE PROCESS OF THE LENS CAPSULE AFTER INJURY*

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Since there seems to be a considerable difference of opinion among oculists as to what happens when the lens capsule is injured and what, if any, is the mechanism of repair, this series of experiments was undertaken, the result of which seemed to be of enough interest to bring to the attention of the Society.

I think it is a common experience that cases that show a large rent of the anterior capsule usually show the entrance of aqueous, with increasing opacity of the lens and often with liquefaction and softening of the lens fibers.

On the other hand, undoubtedly cases have been seen which, when the capsule has been lacerated, have developed an opacity that has remained stationary after the apparent closure of the wound. There are also a few reports in the literature, such as La Rue's, in which a foreign body has passed entirely through the lens, thereby cutting both the

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