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FINAL REPORT

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PROJECT TITLE: The Carbohydrate Metabolism of Ocular Tissues

INSTITUTION AND DEPARTMENT IN
WHICH WORK WAS DONE:

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SUMMARY OF RESEARCH ACCOMPLISHMENTS

1971 - 1972

GENERAL OBJECTIVES

We have been primarily concerned with elucidating the mechanism of cataract formation. The experimental model most widely used in our laboratory has been the sugar cataract, that is, the cataract associated with diabetes and galactosemia. Another model studied by our group has been the cataract produced by radiation. In both types of cataract the crucial phase of cataract development involves changes in the permeability of the lens to cations. As long as the lens is able to prevent the influx of sodium, it is able to withstand the cataractogenic effects. In sugar cataracts, the lens permeability is compromised by the osmotic effects produced by the accumulation of polyols. In radiation cataracts, the ionizing radiation affects the membrane directly, leading to permeability changes. It appears that the lens membranes are affected in these two, and many other types of cataracts as well, during cataract development.

For these reasons, we have studied the role of glutathione and of sulfhydryl groups on the lens membrane, and how they affect cation transport. In addition, we have looked into the calcium effect on aggregation of proteins, since a marked increase in calcium occurs when the lens turns cataractous. We have also continued our studies on the mechanism involved in sugar cataracts.

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Sulphydryl Membrane Effects

The exact role of glutathione (GSH) in the lens has not been elucidated. GSH is present in exceptionally high concentrations in the lens and a loss is reported in sugar and radiation cataracts. To determine the importance of glutathione, a specific oxidant, diamide, was applied to the lens. Rat and rabbit lenses incubated in the absence of glucose with diamide lead to progressive loss in GSH. If glucose is added to the medium the GSH level is maintained at normal levels, presumably by the continuous generation of NADPH through the pentose shunt mechanism. When the lenses are incubated with diamide in the absence of glucose, there is a marked decrease in the uptake of rubidium ions. Studies have revealed that the amount of rubidium is markedly accelerated in the diamide-treated lenses. In the presence of glucose, neither the leakout nor the uptake is significantly altered in the diamide-exposed lenses. These findings lead us to suggest the presence of certain -SH groups on the lens membranes which can undergo oxidation when GSH level is reduced. These membrane -SH groups are involved in the cation uptake process, as well as in determining the cation permeability. Thus the action of glutathione appears to maintain the -SH groups in the reduced form for proper membrane function.

The membrane-SH groups could conceivably be vulnerable to ionizing effects and other oxidizing conditions. For this reason, studies were conducted to determine the effects of various oxidizing agents, such as peroxide, ascorbic acid, and iodosobenzoate.

The results of the effects of ascorbic acid are found in the appended reprint.

The results with peroxide and iodosobenzoate gave similar effects. Striking decrease was observed in the rubidium uptake when the lenses were treated with low levels of these oxidants in the absence of glucose. The addition of glucose in the medium protected against the effects of the oxidant. The rubidium leakout process was less affected than was the uptake process. The striking effect of these oxidants appears to be on Na-K ATPase. A marked lowering was observed on this transport ATPase in the lenses treated by these oxidants. It appears that the experiments with low levels of oxidants of -SH groups indicate that the Na-K ATPase is much more sensitive than the membrane thiols.

Protein Aggregation Studies

A number of investigators have speculated that the loss of transparency associated with cataract formation may be produced by the development of small, irregularly spaced scattering centers whose index of refraction is different from that of the surrounding lens proteins. A condition may exist in the lens that leads to aggregation of proteins. If the number of these aggregates is large enough, or if the molecular weight is of sufficient dimensions, then light-scattering might occur, resulting in a cloudy appearance of the lens.

A study to check these possibilities is underway and the first paper on this subject is appended.

In additional studies, we found that there are aggregates having a molecular weight greater than approximately 150×10^6 gm per mole present in the soluble fraction of both aging normal and cataractous human lenses. This protein population is approximately 5% of the total soluble protein in lenses up to age 75, and increases to 10 - 15% in lenses aged greater than 75 years, and in cataracts. The amino acid composition of the heavy molecular weight soluble aggregates of normal lenses is different in leucine content from alpha, beta and gamma crystallins; and different in tyrosine content from beta and gamma crystallins. The aggregates from cataractous lenses show a greater increase in leucine and greater decrease in tyrosine.

Calcium, an ion which increases in concentration in cataractogenesis, induces alpha crystallin to aggregate, and induces heavy molecular weight fraction to aggregate. This aggregation is irreversible by dialysis or chelation.

Sugar Cataracts

As a result of studies, it now appears that a common mechanism is responsible for cataracts associated with diabetes and galactosemia. The factor that is responsible for triggering the process of cataract formation is the enzyme, aldose reductase. Under normal conditions, the lens aldose reductase is in a relatively inactive or dormant state. However, in galactosemia, a rare hereditary disease in which there is an elevation of galactose in the blood, or in diabetes, in which the elevated blood sugar is glucose, aldose reductase is activated or "turned on." In these conditions the enzyme converts the sugars to sugar alcohols or polyols. The formation of polyols within the lens fibers has a serious adverse consequence. Because polyols are not rapidly metabolized further, nor are they able to diffuse or leak out of the lens cells or fibers, they accumulate to extremely high levels. This causes a hypertonicity that draws water into the lens and leads to osmotic swelling of considerable magnitude. In essence, this is the initial phase in cataract development of diabetes and galactosemia. There are many other changes that occur but all these can be related to the osmotic change, that is the lens swelling caused by the accumulation of polyol. The vacuoles that are visible early in the cataract involve areas of the lens where considerable liquefaction of fibers occurs because of over-hydration. Other chemical changes that are observed can also be explained by the osmotic swelling.

Progress in the understanding of the process of the development and prevention of these cataracts came about not only through animal studies but also by two other types of experimental approaches. One approach was to simulate the conditions of diabetes and galactosemia in vitro and to follow the entire course of development of the cataract in the test tube. It is now possible to take an isolated rat lens, incubate it in the presence of high level of sugar, and develop a cataract. The second lens of the pair is kept in normal medium as a control and its clarity and transparency is maintained several weeks longer than the contralateral lens that develops the cataract. This organ culture technique has proven useful not only in elucidating the mechanism of cataract formation but also in determining the conditions by which cataracts can be prevented.

The second experimental approach that needed to be developed concerned direct ways of preventing these cataracts. Once we were able to understand the basic mechanism involved in the formation of these cataracts the approach to prevent them from occurring became obvious. Since there was every indication that aldose reductase was the initiating factor, it seemed reasonable that cataracts might be prevented by appropriate chemical compounds that would inhibit the action of this enzyme. The Ayerst Laboratories were extremely helpful

in aiding us in our search for compounds capable of inhibiting aldose reductase. The inhibitory action of compounds was first tested against aldose reductase purified from the lens. The compounds that showed inhibitory activity were then used to see if they could prevent cataracts produced in lens culture. Inhibitors which were promising in lens culture were then tested for effectiveness in cataracts produced in experimental animals. We have now uncovered an aldose reductase inhibitor that is capable of preventing cataracts in the galactosemic rats.

1. Jedziniak, J., Kinoshita, J.H., Yates, E.M., Hocker, L.O. and Benedek, G.B.: Calcium-induced aggregation of bovine lens alpha-crystallin. Invest. Ophthal. 11:905-915, 1972.
2. Fukui, H.N., Epstein, D.L. and Kinoshita, J.H.: Ascorbic acid effects on lens rubidium transport. Exp. Eye Res. 15:309-313, 1973.