BIOCHEMICAL STUDIES ON THE OCULAR LENS IN
RELATION TO CATARACTOGENESIS

Final Report

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July 1, 1968 to December 31, 1978

PREPARED FOR THE U. S. ENERGY RESEARCH AND
DEVELOPMENT ADMINISTRATION UNDER CONTRACT
No. E(11-1)-2012-38

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I. SCOPE AND PURPOSE

The scope of these studies was originally limited to determining the effect of ionizing radiations on the ocular lens and devising methods of preventing or arresting the formation of cataracts.

The scope has since been broadened to include studies of all aspects of the chemistry, physiology and cytology of the normal and cataractous lens. The overall purpose is to generate knowledge that will be useful in devising therapy, not only for cataracts produced by radiant energy, but the much more common kind of cataract that occurs in older people and makes cataract second only to glaucoma as the greatest cause of blindness.

II. SUMMARY STATEMENT OF ACCOMPLISHMENTS

As indicated in the scope and purpose of this program, the broad aim of these investigations was to provide an understanding of the mechanisms by which various inorganic ions and a number of organic substances, particularly amino acids, enter and leave the ocular lens and to assay the role of these mechanisms in the physiological and pathological conditions of the eye. The studies also dealt with the mechanisms of glutathione in the lens and its relationship to amino acid transport, the effect of X-ray on the protein aggregation mechanism as well as the studies of the biochemical changes associated with the development of the experimentally induced galactose cataract.

The major findings and accomplishments of these studies during the stated contract period are presented in broad outline form in the following pages; the details of many of these studies have already been published in articles listed at the end of the report or in the appended reprints.

III. OUTLINE OF MAJOR FINDINGS

A. Metabolism and Biochemistry

1. Demonstration that turnover of glutathione in the lens involves total breakdown through the action of peptidase and resynthesis from its constituent amino acids.
2. Demonstration that the lens contains a mechanism for the degradation of glutathione and that the rate of breakdown of the peptide accounts largely for the normal turnover of this peptide in the lens.
3. Evidence that the degradation of glutathione in the ciliary body and the lens may involve the enzymes of the " γ -glutamyl cycle."
4. Evidence for the presence of oxoprolinase, another enzyme of the γ -glutamyl cycle, not previously reported in the lens.
5. Isolation and characterization of the enzyme oxoprolinase from bovine lens epithelium.
6. Observation that the lens contains a significant amount of oxoproline, presumably a normal intermediate of the γ -glutamyl cycle.
7. Oxoproline levels in rabbit lenses cultured in the presence of methionine and a specific inhibitor of oxoprolinase (imidazolidone-carboxylic acid) and in lenses of rats injected with the inhibitor and the amino acid were found to be unaffected. These results are in contrast to those obtained by Meister on the kidney and suggest that either γ -glutamyl cycle is not involved in the transport of amino acids or that metabolism of oxoproline in the lens may be different than that in the kidney.

B. Transport

a) Kinetic Properties

1. Formulation of a mathematical model that describes quantitatively the movement of the alkali metal cations - potassium, rubidium, and cesium - into and out of the lens.

2. Demonstration that potassium, rubidium, and cesium are actively transported into the lens by a carrier-mediated system, similar to or identical with Na-K ATPase, with a single kind of active site.
3. Observation that permeability of the lens for the above mentioned cations is dependent on absorptive rather than frictional forces, i.e. on the anionic field strength of the membrane that limits diffusion.
4. Demonstration that thallium, although in Group III in the periodic table, is actively transported into the lens by the same system responsible for transport of potassium and the other alkali cations, but possesses an affinity for the carrier approximately seven times that of the potassium ion.
5. Characterization of the mechanisms responsible for maintaining high concentrations of the amino acid taurine and inositol in the lens and ciliary body.
6. Evaluation of the transfer coefficients describing the rate of active transport of sodium out of the lens and the rate of movement into rabbit lenses from which it appears that, similar to red blood cells, three sodium ions are actively transported out for each two potassium ions transported into the lens.
7. Supportive evidence that active transport of sodium from the lens is responsible for its bioelectric asymmetry, viz., almost double the voltage and electric current across its anterior compared with posterior surface.
8. Demonstration of transport of reduced glutathione into the lens by a carrier-mediated process that appears to share the same site as cysteine and that peptides in general may be transported into the lens by processes which are energy and temperature dependent.
9. Demonstration that there is a direct link of the transport of taurine and Na-K ATPase in the lens as evidenced by the decrease in the accumulation of taurine when intracellular potassium is reduced and by the immediate effect of ouabain on taurine accumulation.

10. Observation that inositol is transported into both the ciliary body and lens by an active process.
11. Observation that although inositol is transported by a specific carrier-mediated process into both the lens and ciliary body, the transport system is inhibited by several neutral amino acids.
12. Demonstration that the transport system for inositol in the lens and ciliary body, like that of several amino acids, is dependent on the extracellular sodium concentration.
13. Finding that the movement of lithium in the lens is unique in that it is transported by carrier-mediated processes that obey Michaelis-Menten kinetics both into and out of this organ and that potassium is a potent competitive inhibitor of the carrier-mediated transport into the lens, but only weakly inhibits influx of potassium and this by a non-competitive process.
14. The transport of lithium out of the lens does not appear to involve the sodium pump, and its passive exchange is independent of observed differences in bioelectric potential.
15. Difference in the affinity of lithium and other cations for the carrier responsible for its active transport in comparison with the relative permeabilities demonstrated that a common pathway cannot be responsible for the discrimination by cations for both the pump and the leak.
16. Results of chloride flux experiments and agreement between bioelectric potential and distribution ratio of chloride support the conclusion that this anion moves both into and out of the lens solely by passive diffusion.
17. Procedures involving the collection of CO_2 or $^{14}\text{CO}_2$ freed from lenses by acidification have been developed for the quantitative determination of normal or radioactive bicarbonate. The concentration of this anion in lenses removed from rabbit eyes under conditions that minimize loss of CO_2 was found to be 16.8 mM.

18. Demonstration that the transport system responsible for sodium extrusion was carrier-mediated and involved a single site. Moreover, velocity of sodium transport was capable of being stimulated by nearly a factor of three at internal sodium levels of approximately 60 mM.

B. Electrical Properties

1. Evidence, for the first time, of the extent that electric forces affect transport of the alkali cations, increasing influx by about 15 percent and decreasing efflux by over 60 percent.
2. Development of an apparatus and electronic circuitry with which the open circuit voltage and short circuit current can be measured either across the whole lens or from its interior to solutions bathing either its anterior or posterior surface.
3. Determination of the separate contributions of the epithelium and fiber membranes to the short circuit current and the separate resistance of the anterior and posterior bounding membranes.
4. Correlation of the movement of sodium and potassium with the short circuit current across the lens.
5. The existence, origin, and magnitude of an electrogenic potential has been investigated by studying the acute effects of reduced temperature and metabolic inhibitors on short-circuit potentials and ion fluxes in rabbit lenses. Potentials from isolated lenses, determined with glass microelectrodes decreased within ten minutes, ^{following} chilling to 4°C, or poisoning with ouabain (10^{-4} M).
6. A formulation which shows the equality of influx and efflux of sodium and potassium ions in the rabbit lens at steady state was derived on the basis of the pump-leak model of ion transport and used to derive an equation which expresses the electrogenic and diffusional components of the potential difference in terms of active and passive transport coefficients and the cation concentration gradients existing in lenses maintained in culture.

C. Cataract and Aging

1. Observation that although the sorbitol pathway exists in the ciliary body, accumulation of sugar alcohols is not the cause of the reduction of amino acid levels in the aqueous humor of diabetic animals.
2. Demonstration that the loss of amino acids from a lens made cataractous from diabetes is due to impairment of active transport as well as to an increase in permeability of lens membranes as a secondary result of the accumulation of sugar alcohol via the sorbitol pathway.
3. Evidence that glutathione synthesis in galactose cataract is unaffected and is in contrast to the observations in X-ray induced cataracts where the enzymes of GSH synthesis have been shown to be inhibited.
4. Observation that concentrations of free amino acids and glutathione, which decrease rapidly during galactose cataract formation, return to near normal values during the reversal phase of these cataracts despite the persistence of a small pinhead nuclear opacity and continued hydration of the lens.
5. Observation that continued hydration of lens during the reversal phase of galactose cataract is related to the abnormal level of sodium and potassium ions rather than to the presence of dulcitol.
6. During initial and vacuolar stages of galactose cataract formation, the potential decreased in parallel with the decrease in concentration of potassium. The latter results primarily from dilution consequent to an increase in hydration. Throughout these stages, concentrations of sodium and chloride in cataractous lenses increased gradually with time on the galactose ration up to the thirteenth day. The concentration of sodium and chloride rose abruptly by the fifteenth day at which time the concentration of potassium fell abruptly. These changes were parallel to the sudden appearance of nuclear cataract and were accompanied by almost total loss of potential reflecting the disappearance of the electrolyte gradient.
7. Investigations of X-irradiated rabbit lenses have shown that the level of reduced GSH decreased by 75 percent three weeks prior to cataract formation while levels of oxidized GSH and protein-bound GSH remained unaltered, indi-

cating that GSH synthesis has been reduced or that the tripeptide is lost through leakage out of the lens.

8. Cataractous lenses from X-rayed rabbits showed a decrease in the relative amount of β and γ -crystallins and an increase in α -crystallin and high molecular weight protein aggregates.
9. The crystallins released from HMW proteins showed an absence of tyrosine; the significance of the absence of tyrosine is not apparent at this time.
10. The bioelectric potential decreases steadily throughout the initial stages of development, reflecting primarily the increase in membrane permeability to potassium ions.
11. There is observed a significant decline in potassium levels in the aging lens, corresponding to a 40 percent decrease in the rate of potassium transport into the lens.

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