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BIOCHEMICAL STUDIES ON THE OCULAR LENS IN
RELATION TO CATARACTOGENESIS

Progress Report

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pg 9

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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.

ABSTRACT

Further studies were undertaken to determine whether γ -glutamyl cycle is involved in transporting amino acids from aqueous humor into the lens. 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 into the lens or that metabolism of oxoproline in the lens may be different than that in the kidney. Accumulation of several amino acids into the lens was reduced in the presence of Brom cresol green, which is a known inhibitor of γ -glutamyl transpeptidase, an enzyme of the γ -glutamyl cycle, responsible for translocating amino acids into cells. However, it was not possible to conclude that the reduced accumulation of all amino acids was through inhibition of the enzyme since transport of α -AIB, which is not an acceptor for transpeptidase, was also reduced in the lens.

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

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.

Kinetic and bioelectric studies and kinetic investigations of the movement of chloride and its distribution in the rabbit lens have indicated that all movement of this anion occurs passively along electrochemical gradients. Similar studies of kinetics and concentration gradients of bicarbonate ions led to the conclusion that this anion also moves passively into and out of the lens at remarkably rapid rates probably due to the high permeability of the lens membranes to CO_2 .

The development of galactose cataract in lenses from galactose fed rats was investigated to study more accurately changes in active and passive transport of sodium, potassium, and chloride. Results of sodium flux experiments suggest that sodium transport is unimpaired during initial stages of cataract formation, a conclusion consistent with the observation that the level of lens sodium is only slightly elevated. Unlike sodium transport, the turnover rate of potassium increased by a factor of two, while the active accumulation decreased fourfold. In order to evaluate more completely changes in cation and anion concentrations in cataractous lenses, bioelectric potentials were measured and observed to decline gradually over thirteen days, followed by a sudden loss of potential on the fourteenth day when nuclear opacities became visible. Such a fall in potential might contribute to the observed decrease in potassium and increase in chloride since a 50 percent decrease in potential would theoretically alter the passive contribution to the gradients by nearly 30 percent.

Progress Report

STUDIES OF PROTEIN-BOUND GLUTATHIONE IN NORMAL AND CATARACTOUS LENSES

The hypothesis that enzymes of the glutamyl cycle serve a carrier function for amino acid transport into various cells has been further examined. It was previously established (see E(11-1)-2012-26) that all the enzymes of the glutamyl cycle are present in the ciliary body and lens. While these studies are consistent with the aforementioned hypothesis, direct evidence for the participation of the cycle in active transport of amino acids is lacking. One approach to study this problem was to inhibit the enzyme oxoprolinase with a specific inhibitor - imidazolidone carboxylic acid (ICA) - and measure the levels of oxoproline in the tissue. Rabbit lenses and ciliary bodies were cultured in the presence of 1 mM methionine and 5 mM ICA and oxoproline content of the tissues determined. There was no significant difference between the controls and experimental lenses or ciliary bodies; the oxoproline content ($\mu\text{moles/g}$) was found to be $1.34 \pm .405$ in normal lens while experimental lenses showed a value of $1.30 \pm .453$. Similarly, the oxoproline content in the normal ciliary body was $.43 \pm .172$ and in the experimental tissues it was $.41 \pm .110 \mu\text{moles/g}$.

Experiments were also carried out in vivo in which rats and mice were injected with ICA and methionine and oxoproline content determined in the lens. Again there was no difference between the oxoproline content of lenses in injected and control animals. These findings are in contrast to the observations in kidney where oxoproline content of injected animals was signifi-

cantly elevated over the control as reported by Meister.

In another series of experiments we have attempted to inhibit the enzyme γ -glutamyl transpeptidase, which is an acceptor for amino acids and thus may play a role in their translocation into cells. It was found that the accumulation of methionine, arginine, phenylalanine, glycyl-glycine and α -AIB into the lens was inhibited by Brom cresol green. However, since α -AIB is not an acceptor for the γ -glutamyl moiety, the question may be raised whether inhibition of any amino acid transport into the lens by Brom cresol green is indication that γ -glutamyl transpeptidase is involved. A search for more specific inhibitors of γ -glutamyl transpeptidase will be continued during the next grant period in an effort to establish more exactly the role of the γ -glutamyl cycle in amino acid transport in the lens.

STUDIES ON X-IRRADIATED CATARACTS

Preliminary studies have been conducted on the formation of high molecular weight (HMW) protein-aggregates in cataracts in rabbits which developed two to three months after X-irradiation. The level of reduced GSH in irradiated lenses decreased by 75 percent three weeks prior to cataract formation. Analysis of the cataractous lenses showed no significant increase in the levels of oxidized glutathione (GSSG) or protein-bound GSH. This suggests that the decrease in GSH occurring in irradiated lenses is due primarily to a decrease in the rate of GSH synthesis and loss of GSH or GSSG through the lens capsule.

Soluble proteins from control and X-ray induced cataractous lenses were fractionated into α , β and γ -crystallins with the use

of gel filtration chromatography (Sephacrose 6B). Cataractous lenses exhibited a decrease in the proportions of β and γ -crystallins and an increase in α -crystallin and HMW protein (4×10^6 molecular weight). The HMW protein accounted for 15 to 30 percent of total soluble protein in the cataractous lenses. An important observation is that the aggregation process appears to involve more than a single soluble crystallin. Also, disulfide bonds appear to have an important role in the formation of these aggregates in X-ray cataracts. A major thrust during the next project period would be to identify the various crystallins involved as well as determine the type of bonding responsible for the aggregation process and then extend the studies to human cataractous lenses.

ANIONIC COMPOSITION AND KINETICS IN THE LENS

Studies on the mechanism of chloride movement into and out of cultured lenses and the relative concentration of this anion in the different parts of the lens has been extended during the current year. This was accomplished by analyzing chloride fluxes and bioelectric potential on the basis of the pump-leak model of ion transport. Curves showing the efflux and influx of ^{36}Cl have been presented in an earlier ERDA report. It was concluded that all movement of chloride in and out of the lens occurs passively from the close agreement between the rate of accumulation of ^{36}Cl found experimentally and that predicted on the basis of a theoretical curve calculated on such an assumption from a rate constant obtained from the second phase of a triphasic curve obtained by measuring the rate of efflux of ^{36}Cl from lenses preloaded with the radioisotope.

That only passive forces are also involved exclusively in movement of chloride in lenses in vivo was suggested by the agreement between the potential found experimentally in lenses freshly removed from the eyes and the potential calculated from the ratio of the intracellular chloride concentration of lenses to the concentration in the intraocular fluids bathing the lens and that calculated from the Nernst Equation for the chloride equilibrium potential. Details of this investigation are to be found in the accompanying reprint.

A similar study of bicarbonate concentration and kinetics in the lens has also been completed during the past year. This work was undertaken to resolve the uncertainty that exists concerning the bicarbonate between the lens and the aqueous and vitreous humors. This study was undertaken because of the effect of the intracellular concentration of these diffusible electrolytes chloride and bicarbonate on the fixed charges present in the intracellular fluid and the role the latter may play in the control of lens hydration. The exchange rate of bicarbonate in the lens was extremely rapid being exceeded only by that of water, which suggests that the high flux of bicarbonate is determined primarily by the diffusion rate of CO_2 . Details of this investigation are also shown in an accompanying reprint.

BIOELECTRIC POTENTIALS AND GALACTOSE CATARACT

The development of cataract in lenses of galactose fed rats is believed to be initiated by an increase in cation permeability due to osmotic forces produced by dulcitol accumulation in the lens. During the final stages of cataract formation, Kinoshita

reported that membrane leakiness to cations is no longer compensated by an increase in pump activity as he suggests it is in the initial stage, resulting in accumulation of sodium and potassium. To study more accurately changes in active and passive transport during the development of galactose cataract, kinetics of sodium and potassium movement were investigated.

The rates of efflux of sodium in lenses from rats fed a galactose diet for three days did not differ from those rats fed a normal chow ration. These results suggest that the sodium pump is unimpaired during the early stages of the development of galactose cataract. The rate of influx of sodium in lenses removed from galactosemic rats after three days was also the same as that of control lenses. These results might be interpreted as indicating that there is no change in permeability of the ion restricting membranes in the lens during this period since rate of diffusion into the lens along a concentration gradient is a measure of the permeability. However, previous experiments based on the pump-leak model have shown that the potential is also an important factor in determining passive movement of ions in and out of the lens. Accordingly, the potential of lenses from galactose fed rats was also measured and found to be some 30 percent lower than that of control rat lenses. Calculation of the comparative permeability of galactosemic and control lenses, taking into account the effect of the decreased potential, showed that galactose feeding had approximately doubled the permeability of lenses to sodium.

In order to evaluate more completely the changes in cation

and anion concentrations which occur in galactosemic rat lenses, bioelectric potential of lenses in situ from rats fed galactose were measured for the first time over a two week period during cataract development. The potential gradually declined over thirteen days, followed by a sudden and total decline on the fourteenth day when nuclear opacities became visible. Such a fall in potential might be expected to explain partially the observed decrease in potassium concentration and increase in chloride concentration since a 50 percent decrease in the negative potential would theoretically alter the passive contribution to the potassium and chloride gradients in opposite directions, by approximately 30 percent.

SOURCE OF ELECTROGENIC POTENTIAL IN THE OCULAR LENS

Two studies have been completed showing the existence and source of an electrogenic component of the bioelectric potential in the lens. The first of these involved techniques generally employed in electrophysiology to demonstrate the existence of electrogenic pumps, viz. to assume that any depolarization following chilling or exposing tissues to ouabain is electrogenic in origin. When these procedures were applied to rabbit lenses, the potential fell 10 and 19 mV, respectively, and was accompanied by a decrease in the rate of active transport of potassium into the lens and sodium out of it. The rates of passive flux of both ions was unaffected, hence the potential was associated exclusively with movement of sodium and potassium. A preprint of a paper accepted for publication in EXPERIMENTAL EYE RESEARCH describing the results of this investigation is enclosed.

The second investigation was undertaken to establish the magnitude and source of the electrogenic potential in undisturbed lenses. To accomplish this a formulation which expresses the equality of influx and efflux of sodium and potassium ions in the lens at steady state was derived on the basis of the pump-leak model of ion transport. Equation (1), based on an analysis of the steady state fluxes, was developed which relates the bioelectric potential to the active and passive transport coefficients and the concentration gradients existing under these conditions as determined previously in cultured lenses.

$$E = - \frac{RT}{nF} \ln \frac{K_{pNa} [Na]_F - K_{pK} [K]_M + K_{dNa} [Na]_F + K_{dK} [K]_F}{K_{dNa} [Na]_M + K_{dK} [K]_M} \quad (1)$$

$\frac{RT}{nF}$ has the usual significance and subscript F corresponds to lens fiber water, M represents culture media, K_p denotes coefficient of active transport (pump), and K_d denotes coefficient of diffusion.

The electrogenic and diffusional potentials calculated from the equation were found to be -12 and -32 mV, respectively, the sum of which corresponds almost exactly to the total bioelectric potential determined experimentally under similar conditions, viz. -42 mV. The magnitude of the electrogenic potential is in good agreement with that obtained by inhibiting active transport, and arises from the difference in velocity of active extrusion of sodium from the lens and active transport of potassium into it.

The interrelationship of potential and active and passive fluxes of sodium and potassium is shown schematically in Figure 1 where the contribution of active flux to the -12 mV electrogenic component is shown by the solid lines; the passive fluxes

associated with ion gradients responsible for the diffusion potential of -32 mV is shown by the broken lines.

A manuscript presenting a more elaborate mathematical analysis has been submitted for publication, a preprint of which is enclosed.

Progress Report

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July 1, 1975 to May 31, 1977

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