INCORPORATION STUDIES ON THE PROTEINS OF HUMAN NORMAL AND SENILE CATARACTOUS LENSES

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ABSTRACT

The incorporation of labelled amino acid into the lens proteins of different types of senile cataractous and normal human lenses has been studied. Lowered incorporation of labelled amino acid was observed in the mature and hyper mature types of cataracts.

A DECREASE in the total proteins of lenses was found in all types of senile cataractous lenses (Mach[1], Maraini *et al.*[2]). The results of Mach[1] have made it clear that the insolubilization of protein does not account for the decreased amount of soluble protein. Many views have been put forth to explain the loss of soluble proteins in the cataractous process. Burdon-Cooper[3] has observed the hydrolysis of protein during cataractogenesis. The results of Devi[4] showed a loss of proteolytic activity in the cataractous lenses. In 1968 Charlton and Van Heyningen demonstrated the loss of protein through the leakage of the damaged membranes of the lens fibres. On the other hand, Devi[6] showed lowered protein synthesis in the cataractous lenses.

Maraini *et al.*[7] indicated a high incorporation of labelled amino acid into the lens proteins of mature cataracts. The interpretation of these results is considerably complicated because of the number of unknown factors present in the experimental process like the permeability of the fibre membranes, the efficiency of the amino acid concentration, the free amino acid pool within the lens fibres, etc.

As the homogenization diminishes the above mentioned unknown factors, in the present investigation, we used lens homogenates for the studies.

MATERIALS AND METHODS

Human cataractous lenses were obtained after their intracapsular cryoextraction. The cataractous lenses were classified into different types according to the topography of the lens opacification as described by Desai *et al.*[8]. The normal (non-cataractous) lenses were obtained from the eye bank. The lenses were utilized immediately after their collection. The weighed lenses were homogenized at 0+ C in a medium consisting of 7 ml 0·03 M sucrose, 1 ml 0·025 M potassium chloride and 1 ml 0·03 M potassium bicarbonate. One ml of the reaction mixture had 0·5 ml lens homogenate (50 mg/ml), 0·2 ml 0·1 M Tris buffer (pH 8·5), 10 μ moles ATP, 10 μ moles GTP, 5 μ moles Co^{++} and 1 μ Ci L (^{3}H) lysine (Specific activity 1600 mCi/μ mole). After incubation at 37° C, for one hour the reaction was terminated by the addition of 1 ml of ice cold 10% TCA. The precipitate was washed and dried as detailed by Maraini *et al.*[7]. For radio. activity determination, 10 mg of the residue were dissolved in 0·5 ml of 80% formic acid and 0·5 ml of hyamine in a counting vial. Counting was performed in a Liquid Scintillation System ECIL Model: LSS 20, using standard toluene scintillant system. Values are expressed as counts/minute/100 mg dry weight of the lens.

RESULTS AND DISCUSSION

Table I presents the values of the incorporation of labelled amino acid into lens total proteins. While the normal lenses showed a high incorporation, the diseased lenses showed comparatively low incorporation of the labelled amino acid. The incipient and nuclear cataracts have shown a slight lowering in the incorporation of labelled amino acid as compared with the normal lenses. The depletion of the labelled amino acid was greater in the mature and hyper mature cataracts, the hyper mature cataracts showing a much lower incorporation.

TABLE I

Incorporation of L (^3H) lysine into total proteins of lens homogenate

Values are expressed as counts/minute/100 mg dry weight of the lens.

Each value is the mean cf four determinations ± S.D.

Normal lenses (non-cataractous)	Senile cataractous lenses			
	Incipient cataracts	Nuclear cataracts	Mature cataracts	Hyper mature cataracts
21237 ±370	19937 ±330	19217 ±270	19170 ±140	13950 ±130
22416 ±560	18747 ±570	19514 ±460	17437 ±185	14057 ±210
20915 ±430	19042 ±470	18671 ±180	16959 ±200	13437 ±180
21407 ±380	19672 ±275	18349 ±285	17453 ±160	14255 ±150

The preliminary reports of Devi[6] and Maraini *et al.*[7] show considerably lower incorporation of amino acids into the proteins of mature cataracts as compared with the lenses having posterior and nuclear opacities. However, their results have not been obtained from the incorporation of labelled amino acid into the proteins of different types of cataracts because of the insufficient number of early cataractous and normal lenses. In the present investigation, the incorporation of labelled amino acid into the proteins of different types of cataracts reveals a considerable change in the incorporation of labelled amino acid into the proteins of the senile cataracts, this change being higher in the hyper mature cataracts. The lower incorporation of amino acid into the proteins of mature and hyper mature cataracts may be due to a lower turnover of protein synthesis in the severely diseased lenses. Small changes in the incorporation of amino acid into the lens proteins of earlier cataracts could be due to an unaltered protein synthesizing mechanism in these lenses.

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SUCCINIC DEHYDROGENASE ACTIVITY IN *CRYPTOZONA LIGULATA* (FERUSSAC) DURING AESTIVATION

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ABSTRACT

The levels of activity of succinic dehydrogenase in hepatopancreas, mantle and foot tissues of normal, aestivated and naturally aestivating *Cryptozona ligulata* (Ferussac) were studied. Natural aestivating snails and snails induced to aestivation in the laboratory showed decreased enzyme activity over the normal snails. Addition of body fluid and cerebral ganglia extracts of aestivated snails to the homogenates of normal snail tissues effected a significant decrease in the enzyme activity. Possible regulation of enzyme activity by hormonal factors and amino acids has been suggested.

SHELLED pulmonates have been known to pass into a quiescent state of aestivation during dry periods in summer and remain viable for years in the aestivated state[1]. Most of the physiological processes slow down during aestivation. The oxygen uptake falls rapidly and the stored glycogen reserves decline gradually in *Pila virens*, a pulmonate gastropod[2–4]. The levels of activity of ATPase, Succinic dehydrogenase (SDH), Glutamate dehydrogenase and Cytochrome oxidase decrease during aestivation[5,6] in *Pila globosa*. No such studies on aestivation have been reported in the case of *Cryptozoma ligulata* (Ferussac), a terrestrial pulmonate. Since the metabolic and respiratory activity seem to be altered during aestivation it is likely that the body fluids and the nervous system of the

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