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THE GLYCATION OF BOVINE LENS β_L-, β_S- AND γ-CRYSTALLINS DEMONSTRATED BY FROZEN-SECTIONING AND ISOELECTRIC FOCUSING

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Purpose: We like to demonstrate glycation of β_L-, β_S- and γ-crystallins in the microsectioned bovine lens, by isoelectric focusing using three staining methods.
Methods: Four bovine lenses of 1.185±0.131 years were frozen-sectioned into equator and 11 layers. Water-soluble crystallins were separated by isoelectric focusing and stained: 1. with Coomassie Brilliant Blue (CBB); 2. with the lectin (LEC) Concanavalin-A, followed by horse-radish peroxidase (HRP) and diaminobenzidine (DAB); 3. for glycoprotein receptors (REC), HRP and DAB.
Results: Crystallins are separated into: HM-, α_L-, β_H-, β_L-, β_S and γ-crystallins. In the lectin staining only β_L-, β_S- and γ-crystallins were positive, whereas the α_L- and β_H-crystallins were negative. Contrary to the glycated γ-crystallins in the lens nucleus, the β_S- and β_L-crystallins were only glycated in the anterior cortex and to a somewhat lower extent also in the posterior cortical regions. Receptor staining was about 3 times less sensitive than the LEC staining, but results were comparable. The degree of glycation: G_1 = LEC ÷ CBB for γ_T-crystallins is mean 2.40, for β_S-crystallins 0.77 and for β_L-crystallins 0.28.
Conclusions: The degree of glycation of γ-crystallins in the nucleus is 11 times higher than cortical β_L-crystallins and 3.5 times higher than the cortical β_S-crystallins. Application of Carver's model of chaperone activity gives the right interpretation of this study, because only glycated crystallins may fit into the central cavity (Ø 55Å).

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DIPYRIDAMOLE AND RA-642 REDUCE OPACIFICATION IN DIABETIC RAT LENS: IMPLICATION OF FREE RADICAL PRODUCTION

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Purpose. We assessed the effect of dipyridamole, RA-642 and mopidamol (three pyrimido-pyrimidine derivatives), on lenticular opacities in a model of *experimental diabetic cataracts in rats, and its relationships with its inhibitory* effect of free radical production.
Methods. Diabetes was induced by streptozotocin, and manteined by 3 months. 12 mg/Kg/day of dipyridamole (n = 20), RA-642 (n = 20) or mopidamol (n = 20), were administered *per os*. The production of superoxide anions was measured by phenazine methosulphate [PMS]-induced nitroblue tetrazolium [NBT] reduction. Lipid peroxidation was measured as ferrous sulphate and ascorbic acid [FeAs]-induced malondialdehyde [MDA] production. Opacification of rat lens was quantified by a scale from 0 to 4.
Results. All three pyrimido-pyrimidine derivatives caused a statistically significant reduction of opacification in crystalline lens showed a decrease of 81.6%, 78.9% and 1.8% in lens tissue from rats treated with dipyridamole, RA-642 and mopidamol, respectively. Dipyridamole and RA-642 produced a statistically significant inhibition (50% and 64.8%, respectively) of lipid peroxidation as compared with the group of untreated diabetic rats. Mopidamol did not exert any inhibitory effect on lipid peroxidation. There was a statistically significant correlation between opacification of lens and PMS-Induced NBT reduction and FeAs-induced MDA production.
Conclusions. We conclude that the protective effect of dipyridamole and RA-642 from free radical damage to crystalline lens in the model of experimental diabetes used in this study, is the result of the antioxidant action of these compounds. The effect exerted by mopidamol, however, suggest a possible complementary effect of the pyrimido-pyrimidine derivatives through interaction with other mechanisms (e.g., the sorbitol pathway) implicated in the development of cataracts.

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IMPLICATION OF GLUCOSE AND ASCORBATE OXIDATION IN DIABETIC CATARACT DEVELOPMENT

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Purpose: Among other mechanisms it is possible that the presence of oxidizible subtracts and decompartimentalized transition metal may constitute the relevant mechanism in diabetic cataract development. This study was designed to determine if lens membranes from diabetic patients would be more susceptible to peroxidation. The oxidation of low molecular weight subtract such as glucose and ascorbate was also evaluated as a possible source of oxidative damage in the lens.
Methods: Human lens crystalline were isolated and purified by standard procedures. Lens membrane resistance to peroxidation was evaluated by the use a fluorescent probe, parinaric acid. Lipid oxidation was induced by ascorbate/iron and glucose/ copper. Oxidative damage was assessed monitoring loss of endogenous antioxidants such as vitamin E (determined by normal phase HPLC), formation of hydroperoxides (quantified by FOX technique) and formation of TBARS.
Results: Our data suggested that membranes from diabetic cataracts seems to be more prone to peroxidation as compared to senile cataracts. Apparently this increased susceptibility is not related to vitamin E depletion as both types of cataract present the same levels of vitamin E. In vitro experiments have clearly shown that both glucose and ascorbate may autooxidize in the presence of transition metals leading to extensive formation of hydroperoxides, TBARS, and oxidation of parinaric acid. Ascorbate was clearly shown to be more reactive than glucose. It was further observed, in both cases, that oxidation was almost totally inhibited by the presence of chelating agents such as DETAPAC.
Conclusions: The availability of non-chelated transition metals and presence oxidizible substrates seems to be an important factor in determining the extent of oxidative damage. Premature development of diabetic cataract may involve local oxidative stress or a general increased susceptibility to oxidation. Both glucose and ascorbate seem to be good candidates to mediate such damage depending on the nature and amount of available transition metals.

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REFRACTIVE COMPONENTS IN IDDM: A TWIN STUDY.

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Purpose: To investigate the relationship between duration of insulin dependent diabetes mellitus and lens thickness, as well as anterior chamber depth.

Methods: The study is based on the New Danish Twin Register containing 20888 twinpairs, born between 1953 and 1982. Fifteen monozygotic pairs (MZ), 11 dizygotic pairs of same sex (DZss) and 16 dizygotic pairs of opposite sex (DZos) were investigated. At least one twin in each twinpair had insulin dependent diabetes mellitus. A Twin Control Design was used to estimate the influence of duration of diabetes on lens thickness and anterior chamber depth, as measured by ultrasonography.

Results: In MZ twinpairs a highly significant correlation between intrapair difference in duration of diabetes and intrapair difference in lens thickness was found ($r = 0.88$, $p < 0.001$). In the DZ groups the same trends were seen ($r = 0.58$, $p < 0.05$). The intrapair difference in anterior chamber depth decreased with increasing duration of diabetes in MZ twins ($r = 0.85$, $p < 0.005$). In the DZ groups no corresponding relationships were seen.

Conclusions: We found a significant increase in lens thickness and decrease in anterior chamber depth with increasing duration of diabetes among monozygotic twins.