mechanism differs from other protein-secreting pathways in that the biliary secretion apparently bypasses the Golgi apparatus. Mullock *et al.* (*17*) recently provided biochemical support for a vesicular transport mechanism for IgA.

Intravenous injection of polymeric IgA lacking secretory component resulted in biliary secretion of polymeric IgA possessing secretory component (*7*). This indicates that hepatocytes, in a manner similar to that of other mucosal epithelial cells, may synthesize secretory component for use as an IgA receptor. Thus secretory component may be responsible for the cytoplasmic transfer of IgA across hepatocytes and its secretion into the biliary space.

Lemaitre-Coelho *et al.* (*18*) recently demonstrated that serum levels of IgA in rats become markedly elevated after ligation of the common bile duct. This IgA was of the secretory type and was presumed to enter the serum after refluxing into the sinusoidal space. These observations, together with the present results, indicate that hepatobiliary secretion of IgA derived from plasma cells located in the lamina propria of the gut (*19*) and secreted into portal blood may be a major pathway by which secretory immunoglobulin reaches the intestinal lumen (*20*).

RICHARD H. RENSTON
ALBERT L. JONES
WILLIAM D. CHRISTIANSEN
GARY T. HRADEK
Cell Biology Section, Veterans Administration Medical Center, San Francisco, California 94121, and *Departments of Anatomy* and *Medicine* and *Liver Center, University of California, San Francisco 94143*
BRIAN J. UNDERDOWN
Department of Medical Genetics, University of Toronto, Toronto, Ontario, Canada M5S 1A8

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Cross-Linking of Lens Crystallins in a Photodynamic System: A Process Mediated by Singlet Oxygen

Abstract. *In a dye-sensitized photooxidation system, lens crystallin polypeptides become cross-linked, and a blue fluorescence that is associated with the proteins is produced. These changes are similar to those seen in vivo in the aging human lens. Evidence implicating singlet oxygen as the causative agent of the effects in vitro is presented, and the possibility that this species may play a role in aging and cataractogenesis in vivo is discussed.*

A number of changes occur to human lens crystallins during aging and cataractogenesis. Such changes include protein aggregation (*1*), increased production of insoluble protein (*2*, *3*), oxidation of sulfhydryl groups (*4*), production of non-disulfide covalent cross-links between crystallin polypeptides (*5*, *6*), increased pigmentation in the lens nucleus (*5*, *7*), and production of blue fluorescence not attributable to tryptophan (*8*). Considerable effort has been made to implicate

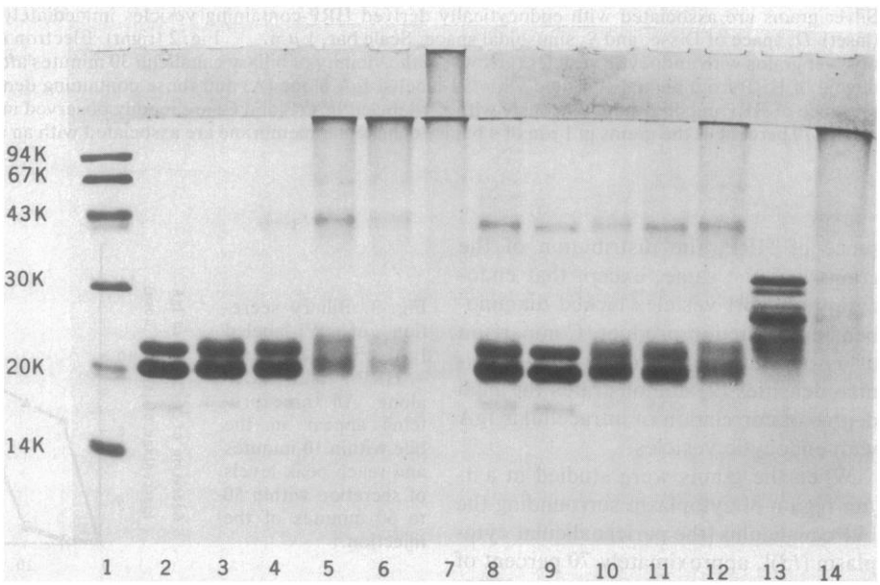

Fig. 1. Polypeptide patterns for bovine lens crystallin samples on a sodium dodecyl sulfate (SDS) polyacrylamide slab gel (4.5 percent stacking gel; 15 percent running gel). All samples were heated for 2 minutes at 100°C in 1 percent SDS and 1 percent 2-mercaptoethanol before being placed on the gel. Sample concentrations were 2.4 mg/ml in phosphate-buffered saline, *p*H 7.5; if present, the methylene blue concentration was $10^{-4}M$. (Lane 1) Standard mixture (Pharmacia) containing phosphorylase b, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, α-lactalbumin. (Lane 2) α-Crystallin normal control. (Lane 3) α-Crystallin dark control. (Lane 4) Deaerated α-crystallin, irradiated 1 hour with methylene blue. (Lane 5) α-Crystallin, irradiated 1 hour with methylene blue. (Lane 6) α-Crystallin, irradiated 2 hours with methylene blue. (Lane 7) α-Crystallin, irradiated 6 hours with methylene blue. (Lane 8) α-Crystallin, irradiated 1 hour with methylene blue and 2 m*M* cyclohexadiene. (Lane 9) α-Crystallin, irradiated 1 hour with methylene blue and 15 m*M* sodium azide. (Lane 10) α-Crystallin, irradiated 1 hour with methylene blue and 2 m*M* glutathione. (Lane 11) α-Crystallin, irradiated 0.5 hour with methylene blue. (Lane 12) α-Crystallin, irradiated 0.5 hour with methylene blue in buffer prepared in D_2O. (Lane 13) Bovine β_H-crystallin, normal control. (Lane 14) Bovine β_H-crystallin irradiated 1.5 hours with methylene blue.

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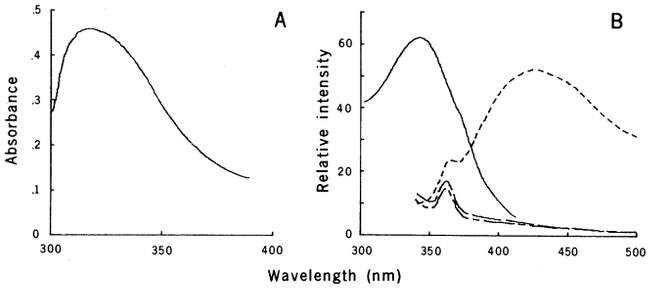

Fig. 2. (A) Difference absorption spectrum (300 to 400 nm) of α-crystallin samples with protein irradiated for 6 hours in the presence of methylene blue ($10^{-5}M$) in the sample beam and protein plus methylene blue kept in the dark in the reference beam. (B) Corrected fluorescence spectra for bovine α-crystallin. The dashed line with the maximum near 430 nm is the emission spectrum for α-crystallin irradiated for 6 hours in the presence of $10^{-5}M$ methylene blue. The two lines below represent the emission spectra for α-crystallin irradiated without methylene blue and α-crystallin plus $10^{-5}M$ methylene blue kept in the dark. Excitation was at 320 nm. The solid line is the excitation spectrum of the sample irradiated with $10^{-5}M$ methylene blue. The emission wavelength was set at 425 nm.

a photooxidative effect as the underlying mechanism of some of these changes, in particular the nondisulfide cross-links, the increased pigmentation, and the blue fluorescence. Photooxidation of tryptophan by near ultraviolet light has received the most attention because it yields pigmented and fluorescent oxidation products (*9*), some of which are present in the lens (*10, 11*). This hypothesis has been questioned (i) because the presumptive effects of such photooxidation are predominantly localized in the lens nucleus, whereas the higher levels of near ultraviolet light reach the anterior cortex, and (ii) because available evidence does not indicate loss of tryptophan from proteins of cataractous lenses (*12*). Another possible mechanism that has been little explored is photodynamic action, or photooxidative effects, produced by exposure to visible light, a photosensitizer, and oxygen.

In order to examine the consequences of photodynamic action on lens crystallins we have irradiated bovine α- and β-crystallins with various photosensitizers. Most photosensitized oxidations proceed via the triplet state of the sensitizer simply because it has a much longer lifetime than the excited singlet state. Two major classes of reaction are available to the triplet sensitizer (*13*). In type 1 reactions, the sensitizer triplet interacts with a reducing substrate to produce free radicals or radical anions. These product radicals can undergo a variety of reactions, including the univalent reduction of molecular oxygen to generate the superoxide anion (O_2^-). The superoxide anion is both a powerful oxidant and reductant and has numerous potential reaction pathways. It can undergo a dismutation reaction to generate hydrogen peroxide (H_2O_2). Furthermore, the interaction of superoxide anion and H_2O_2 may lead to the formation of the hydroxyl radical (OH·). The second reaction mechanism available to the triplet sensitizer is referred to as a type 2 reaction. In type 2 reactions, the sensitizer triplet interacts directly with molecular oxygen. The most common type 2 process involves transfer of energy from the triplet sensitizer to ground state oxygen to produce singlet oxygen ($^1O_2$). Less efficiently, direct electron transfer from sensitizer to oxygen occurs to produce superoxide anion and an oxidized form of the sensitizer. Thus a photodynamic process can be mediated by one or a number of active states of oxygen.

The photosensitizers used in our experiments included riboflavin, methylene blue, and rose bengal. Immediately prior to illumination with 300 to 350 footcandles (1 foot-candle = 1.08 lumen/m^2) of white light from a 15-W Daylight fluorescent bulb, the photosensitizer was added to a solution of lens crystallin (2.4 mg/ml) in phosphate-buffered saline, *p*H 7.5. Experiments were conducted at 37°C. Controls included crystallin plus light without photosensitizer, crystallin plus photosensitizer kept in the dark, and crystallin plus light and photosensitizer under deaerated conditions (four cycles of vacuum for 2 minutes and 95 percent nitrogen, 5 percent carbon dioxide for approximately 10 seconds prior to irradiation).

Portions of the experimental samples were removed after 1, 2, and 6 hours of irradiation. Subunit profiles of the control and experimental samples were determined by sodium dodecyl sulfate (SDS) electrophoresis (*14*). The polypeptide composition of the controls was essentially identical to that of normal α-crystallin (Fig. 1) with subunit molecular weights of 20,000 and 22,000 (*3*). Incubation of α-crystallin with any of the three photosensitizers in the presence of light resulted in dimers and higher multimers of the α-crystallin subunits. These changes did not occur in the dark or in the absence of O_2. Multimers treated with methylene blue were resistant to dissociation by disulfide reducing agents and 7*M* urea. Furthermore, prior treatment of the α-crystallin with *N*-ethylmaleimide under conditions designed to alkylate sulfhydryl groups (*15*) failed to block the subsequent formation of these multimers, a result consistent with formation of nondisulfide, covalent cross-links between polypeptides.

The cross-linking progresses with time. With short periods of irradiation, the major cross-linked species has an apparent molecular weight near 40,000. Weaker bands are sometimes evident in the 60,000- and 80,000-dalton regions. With longer periods of irradiation, aggregates increase in size so that they fail to enter the 15 percent separating gel and ultimately will not enter the 4.5 percent stacking gel.

Our deaerated control demonstrates that the cross-linking process is oxygen dependent. As mentioned above, the interaction of a sensitizer triplet with molecular oxygen can potentially generate four active states of oxygen ($^1O_2$, O_2^-, H_2O_2, and OH·). In order to identify which of these species was involved in the cross-linking of α-crystallin subunits we added to our reaction mixture inhibitors specific for each of the active states of oxygen. We were unable to inhibit cross-linking with superoxide dismutase, catalase, or mannitol, a known hydroxyl radical scavenger (*16*). However, the singlet oxygen quencher sodium azide and the $^1O_2$ scavengers dimethylfuran and 1,3-cyclohexadiene (*17*) almost completely inhibit the formation of cross-linked protein (Fig. 1). Singlet oxygen–mediated processes are enhanced in D_2O relative to H_2O (*18*). An increased rate of α-crystallin cross-linking is observed when H_2O is replaced by D_2O in the buffer (Fig. 1).

Nondisulfide covalently cross-linked protein is associated with a nontryptophan blue fluorescence in the nucleus of aging and cataractous human lenses. This blue fluorescence typically has an excitation wavelength (λ_{max}) of ~ 340 nm and an emission wavelength (λ_{max}) of ~ 420 nm. In addition, the nuclear protein of human cataractous lenses shows an increased absorption between 300 and 400 nm (*19*). When we examined the emission and excitation profiles of bovine α-crystallin irradiated for 6 hours with methylene blue, riboflavin, or rose bengal, an emission λ_{max} of approximately 425 nm and excitation λ_{max} of approximately 340 nm were obtained (Fig. 2). Neither α-crystallin exposed to

light without photosensitizer nor α-crystallin kept in the dark with photosensitizer gave any significant fluorescence at these wavelengths. An increase in absorbance between 300 and 400 nm accompanied development of fluorescence in the photooxidized samples as Fig. 2 demonstrates.

Singlet oxygen-mediated photooxidation has been shown to have damaging effects on proteins (*20*). The principal mechanism of such damage is believed to be via photooxidation of the amino acid residues histidine, tryptophan, tyrosine, cysteine, and methionine (*21*). We have shown above that singlet oxygen has marked effects on bovine α-crystallins. In addition we have found that bovine β-crystallin is more sensitive to the cross-linking process than α-crystallin (see Fig. 1) and that human crystallins appear to be as sensitive as the bovine crystallins. Preliminary studies in our system with other proteins suggest that crystallins may be particularly susceptible to cross-linking.

The components necessary for singlet oxygen generation exist in the lens. Light and oxygen are present and the photosensitizers riboflavin and *N*-formylkynurenine (*22*) have been identified in the lens. Fluorescent derivatives of kynurenine isolated from human lens promote photooxidation of lens proteins in vitro by sunlight with production of increased blue fluorescence and pigmentation (*11*). This increase supports the hypothesis that singlet oxygen may be generated in the human lens and could be a causative factor in the changes associated with aging and nuclear cataractogenesis. The increase in vivo in blue fluorescence, pigmentation, and cross-linking is a very slow process, probably due to the presence of very high concentrations of the endogenous antioxidants glutathione and ascorbic acid in the lens (*23*). Ascorbic acid is a known $^1O_2$ scavenger (*24*), and we have demonstrated that glutathione inhibits cross-linking in our system (see Fig. 1). The fact that these cataractogenic changes occur predominantly in the lens nucleus may reflect the much lower concentrations of antioxidants in this part of the lens (*25*). In addition, since protein turnover is essentially absent in the lens nucleus (*26*), photooxidized protein molecules would tend to accumulate.

JOHN D. GOOSEY
J. SAMUEL ZIGLER, JR.*
JIN H. KINOSHITA
Laboratory of Vision Research, National Eye Institute, Bethesda, Maryland 20205

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* Address correspondence to J.S.Z.

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Methylphenidate and Hyperactivity: Effects on Teacher Behaviors

Abstract. *Teacher interactions with hyperactive and comparison boys were observed during classroom activities. A double-blind, methylphenidate-placebo crossover design was used within the hyperactive group. With no knowledge of any child's diagnosis or drug status, the teacher was more intense and controlling toward hyperactive boys taking placebo than toward either medicated hyperactive boys or comparison boys; her behavior did not differ toward the latter two groups. Discussion focused on the need to consider the broad social ramifications of pharmacologic treatment programs.*

Challengers as well as champions of pediatric psychopharmacology agree that stimulant drugs have detectable and predictable effects on the classroom behaviors of children considered hyperactive. Although serious questions remain about the side effects and long-term outcomes of stimulant treatment during childhood, short-term gains for many children have been documented conclusively in double-blind, placebo-controlled studies (*1, 2*).

Almost nothing is known, however, about the social ramifications of methylphenidate—the impact of medication on a child's interpersonal environments. Recent laboratory studies indicate that children's medication influences maternal behavior patterns (*3*). It is important to assess the impact of stimulant medication on a teacher's behavior toward a child, as hyperactive children often have their greatest difficulties—and show the most dramatic medication-related improvements—in school environments (*4*). Further, information is needed about the complex interplay between teacher and child because teachers often serve as the primary evaluators of drug treatment. The present study addressed this question in a quasi-naturalistic classroom setting.

This study was part of an intensive assessment of hyperactive boys taking methylphenidate. In order to create a quasi-naturalistic environment, two 5-week morning enrichment programs were conducted during the summer months. Within each of the two identical summer sessions, there was a Monday-Wednesday class and a Tuesday-Thursday class, yielding four cohorts in all, each with 15 or 16 boys. The boys participated in laboratory components and field trips on the days they were not in class.

Twenty-two hyperactive boys were recruited through local pediatricians. All boys accepted for these studies were previously considered hyperactive by the referring physician, had no other

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