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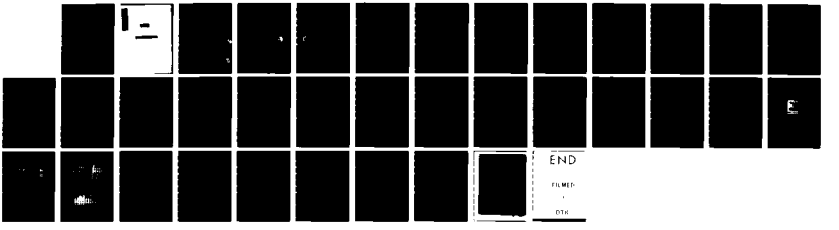

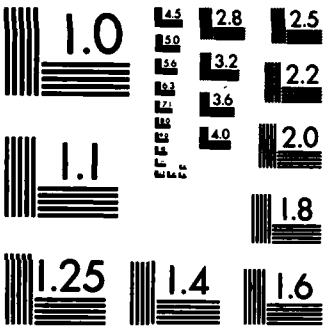

August 1980

Fort Detrick, Frederick, MD 21701

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THE ORIGIN OF CATARACTS IN THE LENS FROM INFRARED LASER RADIATION

Final Report

M. L. Wolbarsht

Supported by
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The cataracts resulting from IR exposure are compared with those linked to UV exposure. The IR exposure produces changes in the lens proteins, the crystallins, while UV exposure seems to attack specific amino acids. Gel electrophoresis of lens proteins have been used to detect the earliest changes possible in cataract formation following exposure to IR from broad band and laser source irradiation. Cataracts can be easily formed in rabbit lenses in vivo when the laser radiation is restricted to the lens alone at power levels above 1 W for 1 minute. Lower

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power levels do not produce immediate cataracts but changes in lens proteins can be detected by thin layer isoelectric electrophoresis of plain polyacrylamide gels and with sodium dodecyl sulfate (SDS) or 6 M urea. The plain gels (pH 3.5 to 10) showed a decrease in the α crystallins indicating a possible change of soluble α crystallin to an insoluble high molecular (HM) weight form. However, small amounts of β and γ crystallins may also be involved in the formation of a HM insoluble aggregate. Soluble HM weight crystallins often were detected as the α crystallin disappeared. This HM soluble fraction may be an intermediate step in the process in forming insoluble α crystallin. Following higher laser power levels the β crystallin has a markedly decreased mobility which also might be a precursor for the insolubilization of all crystallins. These changes are compared with similar changes in the lens proteins which follow broadband IR exposure in vivo, or in vitro, or in lenses incubated in vivo at various temperatures which showed some, but not all, of the same changes. IR laser exposures at power levels above 0.1W can be considered an acceleration of the aging process.

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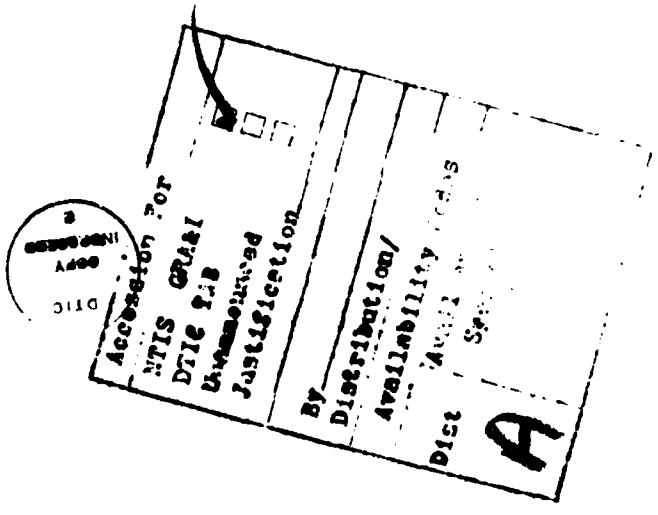

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INTRODUCTION

The transparency of the ocular lens can be disturbed by a variety of different agents resulting in direct cataract formation. Among these agents are the various portions of the electromagnetic spectrum including the ultraviolet, microwave and infrared regions. Ultraviolet cataractogenesis has been the object of much work, and as a result the mechanisms involved are understood comparatively well. On the other hand, even though earlier studies have indicated that the so-called "glass-blower's cataracts" are probably produced by long exposure to infrared radiation, the physical and chemical steps in infrared cataractogenesis are still obscure.

It is well known that almost any disturbances in the eye can cause an increased susceptibility to cataract formation. Thus, any preexisting (or acquired) eye pathology may synergize with the deleterious effects of chronic exposure to infrared radiation. This multi-factorial type of interaction make the interpretation of the data from epidemiological studies of industrial exposures to infrared radiation much more difficult. Nevertheless, such epidemiological studies are still quite valuable in possibly linking some types of preexisting eye pathology to infrared cataractogenesis.

The actual mechanism of infrared cataractogenesis is not clearly formulated at present, so much so that even the initial step is in question. One widely accepted theory holds that infrared radiation is absorbed by the iris (possibly in the pigment granules) and then converted into heat which is conducted to the adjacent epithelium of the lens (Goldmann, 1933; Langley et al., 1960). By contrast, another model is based on direct absorption by the lens, possibly resulting in photochemical degradation of a target molecule or subunit of a lens protein (Vogt, 1930; Vogt, 1932; Wolbarsht et al., 1977).

Glassblower's cataract results from long time industrial exposure (often over a period of years) to low levels of infrared radiation. In these exposures the calculated rise in temperature of the iris and adjacent anterior portion of the lens would be slightly less than 1° C. Goldmann and others have suggested that even this increase in temperature of the anterior portion of the lens in contact with the heated iris will, in time, produce denaturation of a sufficient amount of protein to initiate cataract (reviewed in Wolbarsht, 1978). This denaturation process can be compared with the supposed formation of senile cataract in which there is a loss of ascorbic acid, decrease in total lens protein, and a decrease in the relative amount of soluble protein mostly of the α crystalline moiety. Nevertheless, this model for cataracts resulting from chronic IR exposures to the iris, with purely thermal heat conduction from the iris to the lens, seems unlikely as a cause of cataract. If this were the course of events, then even the elevated body temperatures (38° - 40° C) recorded during many illnesses should be sufficient to initiate cataract formation. Yet, such an effect has not been described. Direct absorption of the radiant energy by the lens would also be unlikely to produce sufficient temperature rise to initiate cataract formation. The lower absorption coefficient of the lens as compared with the iris would indicate the lens would be warmest always behind the iris unless there was significant focussing within the lens. On the other hand, if

direct absorption of the radiant energy by the lens is implicated in cataract formation, then some target molecule(s) must be hypothesized.

Any theory involving possible mechanisms in which the infrared is absorbed directly by the lens to initiate cataract formation needs careful examination. Absorption by the rabbit lens is low in the near infrared but at 1300 nm it becomes abruptly high. This is shown in Figure 1. At 1060 nm (the wavelength of the neodymium laser) the absorption is approximately 20%. Even though the iris pigment, melanin, absorbs only very slightly at wavelengths greater than 1000 nm (Hayes and Wolbarsht 1968; Blois, 1969) as shown in Figure 2, the total IR absorption by the lens still much lower than that of the iris. The relative absorption of the iris and lens at 1060 nm is borne out by their relative threshold to thermal injury by high intensity Nd laser (1060 nm) pulses (Dannheim and Rassow, 1977). The lens requires approximately 30 times as much energy as the iris. Thus, any rise in temperature in the lens developed as a result of chronic exposure would certainly be less by direct absorption than by conduction from the nearby and more highly absorbing iris. However, in either case, the rise should be small. It seems more likely that the temperature rise would stimulate (or inhibit) some specific IR photo-activated chemical process rather than act by an overall increase in metabolism of all the lens constituents.

The target molecule involved in infrared cataractogenesis would not seem to be the same as that involved in the formation of the UV induced senile or brunescent cataract. The development of the brunescent cataract has been studied intensively (see Wolbarsht, 1978 for review). Although there is not a complete agreement on all the steps, the major points, including a link with prior exposure to UV, are commonly accepted. For that reason it is worth describing the steps in brunescent cataract formation here to be able to contrast them with the current data on infrared cataractogenesis.

Brunescent (senile) cataract is characterized by a loss of ascorbic acid, a build-up of glutathione, a decrease in total lens protein, mostly in the high molecular weight moiety (Davson, 1962). In particular the soluble α crystallin decreases as the insoluble albuminoid protein fraction increases (Dische et al., 1956). This reciprocal relationship has been rationalized by showing a common endogenic structure (Woods et al., 1933). However, conversion of the soluble α crystallin to an insoluble albuminoid form is not the first step of the process. The exact nature of the conversion of the soluble α crystallin to the insoluble albuminoid is not known, but it has been suggested that the conversion may involve a conformational change in the portion of the protein that masks the sulfhydryl group (Merola and Kinoshita, 1957). Also, a complex may be formed between trace amounts of glycoprotein components and soluble α crystallin (Dische, 1965). However, as similar changes occur in X-ray induced cataracts, thermally activated (glassblower's) cataracts, nutritionally induced cataracts, as well as in UV cataracts, it seems even more obvious that neither the unmasking of the sulfhydryl group, nor the complexing of the α crystallin with glycoproteins, can be the initial step, but are both triggered by some earlier event.

Previous work by Zigman (1971) and Kurzel et al. (1973a) points to

a possible initial event of the photo-oxidation of tryptophan upon exposure to UV. This is probably the most susceptible amino acid to UV degradation as its excited electronic states s_1 and g_1 are the lowest of all the chromophoric amino acid residues in the lens protein. In this connection it should be noted that the lowest excited state, t_1, of tryptophan has a relatively long lifetime. Other aromatic amino acids, such as tyrosine, phenylalanine and histidine, whose lowest excited states are at higher energy levels will probably transfer any energy absorbed by them to tryptophan. Transfers of energy in this way would maintain tryptophan in its lowest excited state for relatively long periods of time. For this reason the portion of the protein with tryptophan has a high probability of acting as an active intermediate site for secondary photochemical reactions, possibly further photo-oxidation.

Subsequently it has been established by several groups (Kurzel et al., 1973b; Dilley and Pirie, 1974; van Heyningen, 1973) that brunescent senile cataract appears to be identical to the chronic form of the UV induced cataract. This supports the hypothesis that brunescent cataracts are initiated by long term exposure to environmental ultraviolet radiation. Either sunlight or high level UV, artificial light sources could act as the trigger. The adult human lens in vivo is not normally subjected to radiation longer than 2,000 nm or shorter than 293 nm, as the cornea absorbs very strongly in these regions. The lens absorbs most of the radiation that reaches it in the near ultraviolet and in the infrared between 1,100 and 1,400 nm. In the visible range the lens is normally clear and non-absorbing. At the present we have no hint of the relative effectiveness of the near infrared wavelengths. The relative quantum efficiency for cataractogenesis is probably directly related to the photon energy with the more energetic photons the more effective. However, the action spectrum will most likely show that the less energetic photons in the region of 1,100 - 1,400 nm will be the most effective as the lens absorbs the most strongly in this region.

In the brunescent cataract, which develops following exposure to ultraviolet light, several degradation steps in the photooxidation of tryptophan can be detected, such as kynurenine, 3-hydroxykynurenine and certain hydrolyzed products of kynurenine. If similar degradation products in the infrared induced cataract were detected by ultraviolet fluorescence techniques (Kurzel et al., 1973a, b) then UV and IR cataracts would be similar.

Glycoproteins have been implicated in senile cataract formation (Carlin and Cotlier, 1971) on glycosidase activity in lenses; the involvement of the excited state of the glucoside of 3-hydroxykynurenine may be a possibility. Glycoproteins may act as the source for the "active glucose" molecules postulated by Spiro and Spiro (1968) and found by Spector (1971) to be involved with the onset of γ crystallin aggregate formation. Glucose was found to be bound in some fashion to the lower molecular weight γ crystallin components to form longer albuminoid moieties.

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promul-gated by the Committee on the Guide for Laboratory Animal, Resources, National Academy of Sciences-National Research Council.

EXPERIMENTAL METHODS

I. In Vivo Laser Exposures

All laser exposures were made with a CW neodymium-YAG laser (Holobeam Model 250). It is a multi-mode CW laser with the majority of its output at 1.065 μm in an approximately 3 mm diameter beam. The beam was enlarged in collimated form to 24 mm by means of an 8X beam expander (Edmund Scientific). The power input was measured by a Scientech (Boulder, Colorado, Model 360) disc calorimeter which samples the back beam of the laser. The ratio of the front to back beam of the laser output was measured periodically so that the front beam output could be accurately calculated by measuring the back beam power with an appropriate correction factor. The disc calorimeter was calibrated absolutely by passing a known current through a built-in heating resistor while noting the calorimeter output. The measurements of laser power were relatively unaffected by the back reflection from the beam expander which had a constant 10% total air-glass reflection from all optical surfaces.

In general, the right eye was exposed with a beam which impinged on the iris. The beam exposing to the left eye was positioned so that none of the radiation fell on the iris, but passed into the clear portion of the lens. A selected exposure level with a 3 mm beam was given to each eye twice with approximately a minute for each exposure with one day between exposures. Pigmented rabbits were used in most experiments, although some white unpigmented rabbits were used for control runs to indicate whether pigmentation of the rabbit played an important part. No significant differences between white and pigmented rabbits were seen in the lenses from the left eye which were irradiated alone. However, the pigmented irises produced greater lenticular reactions behind them than from the unpigmented ones at similar exposure levels. The rabbits were 1 to 1.5 Kg and 8 wks old. They were anesthetized with 0.6 cc sodium pentobarbitol before exposure.

The pigmented rabbits were anesthetized with paraldehyde intraperitoneally. The eye ball and cornea were anesthetized with Alacaine, and Duke Mix dilator (phenylephrine and mydriasil; 1:1) was used to retract the iris. An eyelid retractor with aluminum foil backed by wet gauze curved as a reflective coating to protect the eyelids and the surrounding areas from burns. A drip system of salt solution prevented extensive drying of the cornea during irradiation.

Following irradiation, the animal was sacrificed, the eyes removed and the lens extracted. Each lens was homogenized in 3.5 ml H_2O, centrifuged at 9,000 rpm and the supernatant containing the water soluble lens protein saved for analysis by gel electrophoresis.

II. Details of Lens Incubation

Lens from four month old pigmented rabbits were used in most experiments. The animals were killed by injecting 4 cc sodium pentobarbitol i.v., and the eyes were immediately enucleated with eye scissors. Each eye was opened posteriorly with a scalpel. Then cuts were made over to the

limbus along four meridians, thus sectioning the sclera into four leaves. These were then pulled forward and laid flat, exposing the vitreous and underlying lens. After removing as much of the vitreous as possible, and cutting peri-limbally through the ciliary process, the root of the iris, and the peripheral cornea, the lens was then gently rolled free of the adherent tissue.

Some lenses were incubated whole. Care was taken to prevent any instruments from touching these lenses. The cleaned lens was immediately transferred to a culture dish filled with culture medium. The culture medium contained 50 parts of TC 199, 20.5 parts of bicarbonate buffer (Merola et al., 1960) and 9.5 parts of solution containing sufficient glucose, fructose, and calcium chloride to achieve a final concentration of 5 mm, 30 mm, and 1.5 mm respectively (Thoft and Kinoshita, 1965). The medium filled dish was placed in a CO_2 incubator under 5% CO_2 at the temperatures indicated in the experimental protocol. Those ranged from normal body temperature to 45° C. The culture was changed every other day, and a sample lens was removed and frozen in distilled water each day. Each lens was homogenized for analysis in 3.5 ml distilled water and centrifuged at 9,000 rpm for 15 minutes. The supernatant containing the water soluble lens protein was placed on SDS gel for electrophoresis.

Other lenses were homogenized before incubation in physiological saline. These homogenates were heated for up to 24 hr in a water bath at temperatures ranging from 37° to 45° C. No significant changes were seen below 40° C. The gel electrophoresis analyses were done at different times, some at half an hour, some at 12 hr, others were continued for the whole 24 hr. No significant precipitate occurred in any of the samples, but they did seem to become more opalescent or cloudy with the longer period of incubation. In order to determine which protein fractions of the lens were changed by incubation, calibrations were established with purified isolated lens crystallins that were available. These include the α, β_H, β_L, and γ crystallins. All were analyzed by the gel electrophoresis isoelectric focusing technique as established by Zigler (1975). Both homogenized and whole lenses were tested as there are indications that the structural integrity of the lens prevents some changes from taking place which are observed in the homogenates. Measurements were also attempted on some lenses for ascorbic acid in a similar fashion to Weiter et al (1975) who incubated rabbit lenses during microwave exposures.

Samples of various purified lens protein were also incubated individually and in various combinations to determine: (1) the effects of incubation at various temperatures on individual proteins, (2) any interaction among the purified proteins to either stabilized or synergized degradation. The purified lens proteins were also tested in buffered solutions in the following pH ranges: 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0. These samples were all heated at 44° C for 22 hr.

Some of the initial tests were made on rat and calf lenses as they were available in large numbers and they have been used extensively by others. Much data on their lens crystallins is available. All results

were checked later with rabbit lenses. For those experiments the calf lenses were obtained from a local abattoir, chilled, and then homogenized in physiological saline. The rat lenses were obtained from the animals in the same way as from the rabbits described above. Some lenses from human eyes (obtained from the eye bank) were tested, also.

III. Electrophoresis

A. Gel Electrophoresis

We used the procedures described by Zigler (1975). Briefly, the excised lenses were placed in cold distilled water, and immediately homogenized with a Willems Polytron (Brinkman Instruments). All insoluble material was removed from the lens homogenates by centrifugation at 27.00 g for 20 minutes at 4° C. The LKB 2117 Multiphor (LKB Instruments, Inc.) was used for isoelectric focused thin layer gel electrophoresis on polyacrylamide slab gels. Special, narrow range gels (pH 5-8) were prepared by the procedure recommended by LKB and were photopolymerized. For a broader spectrum, wide range gels (pH 3.5-10) were used, generally of the preformed types (LKB Pag Plates). Lens samples (approximately 2 mg/ml concentration) from small applicator strips of Whatman 3 mm filter paper were applied to the surface of the gel midway between the anode and cathode. Initially the amperage was set at 50 mA. As the current dropped during focusing, the voltage was increased, but the maximum power was held below 30 W. Between one and one-half to three hours the current stabilized, signaling equilibrium conditions. After an additional 20 minutes to allow the slower moving proteins to complete migration to their isoelectric points, the run was halted.

The gel was removed and stained directly for 20 minutes at 60° C with a standard staining mixture: 75 ml methanol, 155 ml water, 0.25 g Coomassie Brilliant Blue R, 7.5 g sulfosalicylic acid, and 25 g trichloroacetic acid. The plate was then destained overnight at room temperature in a solution containing 1950 ml water, 750 ml ethanol and 240 ml glacial acetic acid. The initial standardization of the technique used frozen calf eyes from Pel-Freeze Biologicals, Inc. All test lenses were analyzed by the same procedure.

B. Urea-Polyacrylamide Electrophoresis

Gels with a final concentration of approximately 6 M urea were prepared from stock solutions identical to those used for standard thinlayer gels except that all stock solutions were made up in freshly deionized 8 M urea instead of water. The same stock electrophoresis buffer (tris-glycine, pH 8.3) was also used. However, in this case one part stock buffer was diluted with three parts 8 M urea. Dithiothreitol was added to the diluted buffer to a concentration of 100 mg/l. Deionization of 8 M urea was accomplished by passing the 8 M urea over a column (3 x 35 cm) of the mixed resin AG 501-X8 (Bio Rad Laboratories). The electrophoretic apparatus and the mechanical techniques in preparing and handling the gels and sample solutions were identical to those used for standard thin-layer gels, except that sample protein solutions were prepared in

the urea electrophoresis buffer described above. Gels were stained in Coomassie Brilliant Blue R, prepared by a one to twenty dilution in 5% sulfosalicylic acid - 5% TCA solution of one percent aqueous Coomassie Blue.

C. Electrophoresis in Sodium Dodecyl Sulfate (SDS)

Electrophoresis in the presence of sodium dodecyl sulfate (SDS) was performed according to the method of Weber and Osborn (1969) with certain modifications. It has been demonstrated that SDS, which is an anionic detergent, denatures proteins to their constituent polypeptides and that the electrophoretic mobility of SDS-denatured polypeptides is linearly related to molecular weight. SDS was obtained from Fisher Scientific Company and was used without further purification. The gel buffer was prepared as follows: 3.9 g $NaH_2PO_4.H_2O$, 19.3 g $Na_2HPO_4.H_2O$, and 2.0 g SDS dissolved in water to a volume of one liter. Before use, the gel buffer was diluted by addition of an equal volume of water. A stock solution for 10% acrylamide gels was prepared by dissolving 22.2 g of acrylamide and 0.6 g methylene bisacrylamide in water to give 100 ml of solution. This stock solution was stored at 5° C in a brown bottle. Staining solution was prepared by dissolving 1.25 g Coomassie Brilliant Blue in a mixture of 454 ml of 50% methanol and 46 ml of glacial acetic acid. The destaining solution contained 75 ml of glacial acetic acid, 50 ml of methanol and 875 ml of water. To prepare twelve SDS gels, 15 ml of undiluted gel buffer, 13.5 ml of acrylamide stock solution, 1.5 ml ammonium persulfate solution (10 mg/ml) and 0.045 ml TEMED were mixed and then pipetted into the gel tubes, 5 mm in internal diameter and approximately 14 cm in length.

Samples were prepared by adjusting the protein concentrations to approximately 1 mg/ml in 0.01M sodium phosphate buffer, pH 7.2, 1% in SDS, and 1% in 2-mercaptoethanol. Before applying samples to the gels they were placed in a boiling water bath for several minutes to insure complete denaturation. Samples were applied to the gels as outlined above and were electrophoresed at 4.5 mA per gel for 4 to 6 hours. After completion of the run the gels were removed from the tubes and cut through the bromophenol blue tracking band. The bottom parts of the gels were discarded and the top sections were left overnight in the following solution: 227 ml methanol, 227 ml water, and 46 ml glacial acetic acid. This solution is identical to the staining solution except that the Coomassie Blue is omitted. This step was added to the procedure to leach out unbound SDS from the gels in order to facilitate staining. The gels were then transferred to tubes containing staining solution for approximately 45 minutes after which they were rinsed and placed in destaining solution in a 37° C oven. Destaining was complete after several changes of destaining solution.

IV. Amino Acid Analysis

The tests for changes in amino acid levels used conventional gas chromatographic techniques. Initially the lens samples were hydrolyzed by heating 6 N HCl at 105° C in sealed evacuated tubes. Each sample was separated in two parts: one was heated for 24 hours, and the other for

48 hours in order to correct for the partial destruction of certain residues and to allow time for complete hydrolysis of hydrophobic amino acids. The values of valine and isoleucin were normally higher in the 48 hour hydrolysates; these values were therefore the more accurate. The test samples hydrolyzed for 72 hours showed no changes from the 48 hour values, thus long hydrolysis did not seem to be required in order to get accurate and reproducible values. As threonine and serine were progressively degraded by this procedure, their values were obtained by extrapolation back to zero hydrolysis time.

The amino acids were converted into their volative derivatives by the following procedures. The hydrolyzed lenses were dried in a desiccator and transferred in 0.1 N HCl to Mini-aktor tubes (Regis Chemical Co.). The HCl was evaporated, and the hydrolyzed amino acids were converted into their n-trifluoroacetyl n-butyl esters by a variation of the method of Roach and Gerke (1969). 0.2 ml of 3 N HCl n-butyl were added to dry sample tubes, which were closed and heated to 100° C for 20 minutes. After cooling the tube, it was opened and the sample again dried. Finally 0.15 ml of 25% (volumetric) trifluoroaceticanhydride in methine fluoride was added to each tube which was sealed and heated to 150° C for 15 minutes. This procedure butylates the carboxyl groups of the amino acids while the amino groups had been trifluoroacetylated. The nonvolatile amino acids were thus converted to volatile derivatives which could be separated with a gas chromatograph.

A Beckman CG 65 gas chromatograph equipped with a disc integrator and fitted with dual glass columns of 1/8" I.D. was used. One column was 6 feet long and was packed with Tabsorb (Regis Chemical Co.). The oven temperature was programmed to rise from 70° to 225° at a rate of 10° per minute. This allowed resolution of 16 amino acids. The second column was 3 1/2 feet long packed with Tabsorb-HAC (Regis Chemical Co.) and was used to quantitate the basic amino acids arginine and histidine. Relative molar response factors were determined for each amino acid by using the methods described above on a standard amino acid mixture with a amino acid isobutyric acid as internal standard. The molar response factors were determined concomitantly with each group of analyses in order to control possible variations associated with the conversion of the amino acids to their volatile derivatives or in the gas chromatograph itself. The amino acid compositions were determined as relative mole percent residues per 100 by dividing each amino acid integrator count by the appropriate response factor, summing the resulting values and finally deriving the total sum of each individual value. Some quicker procedures have been found more reliable during this present period, such as gel electrophoresis, and in other cases new techniques were tried, such as electron probe x-ray microanalysis. Therefore, only relatively few amino acid analyses were done in order to determine agreement with earlier values recorded during previous periods so as to allow a comparison of present experimental techniques with earlier work.

V. Electron Probe X-Ray Microanalysis

The concentrations of certain trace elements in the lens are known to increase or decrease with the formation of a cataract. For example,

normal human lenses are high in zinc; this level is reduced by a factor of 4 in senile cataracts. However, senile cataracts are high in cobalt and iron, and especially high in copper, which was nearly 12 times the normal level. The reduction in zinc and elevation of copper in senile cataracts appear significant in view of the opposite trends for the levels of these trace elements in blood from these same patients. An increase in calcium accompanies degenerative changes in the lens such as normal aging, sclerosis, and cataract formation. In sclerotic lenses calcium may make up over 3% of the ash but in cataractous lenses having the same degree of sclerosis, the value rises to 5%.

The mechanism of cataract formation may be linked to shifts in the lens inorganic constituents. However, in order to test this relation the location and concentration of the various trace elements must be found. The specific location of these elemental shifts within the lens can be monitored by electron probe analysis, often called energy dispersive x-ray analysis (EDX). This analysis is performed by discrimination of the x-rays generated in the specimen lens by the illuminating electron beam of the microscope. The techniques of x-ray microanalysis allows the measurement of both the wavelengths and intensities of the x-ray lines generates a spectrum. Furthermore, it is possible to generate an x-ray map for a specified element and by correlating this with a scanning transmission electron microscope (STEM) map of the same region determine the precise location of the element. Changes in concentration and location of elements can thus reliably be detected.

In this technique whole lenses are excised and are placed directly into 2.5% gluteraldehyde buffered with 0.1 M cacodylate (room temperature, pH 7.4) for 1 hour. Following this primary fixation the specimens are placed in increasing concentrations of buffered gluteraldehyde. The 5, 10, and 25% gluteraldehyde had 5 min fixation time, while the 50% had 15 min. Undiluted Epon 812 was then introduced and after 1 hour replaced by the resin mixture without accelerator (left overnight at 4° C). Capsules were made on the next day from resin mixture with accelerator and were cured in a 60° C oven for 2 to 3 days. This direct gluteraldehyde-to-Epon technique has the advantage of being rapid and simple and minimizes the translocations of elements which occur with the conventional use of dehydration in graded alcohols prior to embedding.

The Epon embedded lenses were sectioned at a thickness of 5 μm, placed on gold grids, and lightly carbon coated. Both cataract and control specimens were examined at 20 KV on an ETEC scanning electron microscope with a KEVEX 5100 Si (Li) energy dispersive x-ray analyzer (Kevex Corporation, Burlingame, CA).

Galactose cataracts in rats were used to standardize the electron probe x-ray microanalytical techniques. Rats (50 g) were fed a diet rich in galactose (1250 g galactose, 250 g casein, 250 g dry milk, 125 g butter, 37.5 g calcium carbonate, 25 g sodium chloride, 750 g graham flour) in order to induce cataract formation. After 10-14 days of galactose feeding, a central cataract appeared and enlarged to fill most of the capsule.

RESULTS AND DISCUSSION

Several techniques have been used to quantify the process of formation of cataracts by exposure to infrared radiation. The results from them can be conveniently described under two headings:

1) Measurements of the radiation necessary to produce visible cataracts in the whole lens in vivo or in vitro from exposure to laser radiation at 1060 nm.

2) Analysis of the lens or its components after exposure to the same type of infrared radiation (with or without changes in the lens temperature during exposure) to determine if precursors could be detected before actual cataract formation could be observed.

As some chemical changes in the lens could be detected long before any cataracts were visible, the main thrust was to select those precursors which were elicited by the smallest exposures. This was in situations where exposure of the lens to higher power levels for the same duration, or the same power level for longer duration exposures would produce visible cataracts. The results from these tests strongly supported the hypothesis that the chemical changes detected were truly precursors of cataractogenesis.

The results of exposure leading to frank cataract formation will be presented first, then the results of the attempts to find precursors. We used many techniques which did not reliably detect any precursor type changes following IR exposure. These tests are described only to indicate the possible lack of value of those analytical techniques in future investigations of IR cataractogenesis.

I. Visible Effects Of Infrared Laser Exposure On The Lens

A. In Vivo

Both brown pigmented and white unpigmented rabbits were exposed to a CW-neodymium laser at 1.06 μm. In some animals the iris was fully dilated, in others the iris was contracted during exposure. When the iris was dilated, the laser beam was confined to the lens. In the undilated cases, a portion of the beam fell directly on the iris surrounding the pupil which was filled with radiation. Thus, the lens was radiated directly through the pupil, and indirectly through the iris.

Cataracts formed rapidly behind the iris of the brown pigmented rabbits following exposures with a 3 mm beam at 14.2 W/cm^2 at the cornea for 2 periods of 60 seconds each 1 day apart. Similar power levels of radiation delivered to the lens did not produce cataracts immediately. Although some cloudiness was occasionally seen following direct radiation of the lens, this cleared within a few hours. Iris exposure of the white unpigmented rabbits at these power levels did not produce observable cataracts. The cataracts which formed from iris exposure in the brown pigmented rabbits appeared directly behind the iris, and in general,

were confined to that region. This cataract formation was apparently the result of heat conduction from the site of absorption in the iris to the lens. Exposures to higher power levels with a 3 mm beam of 21.2 W/cm^2 at the cornea produced cataracts both behind the iris, and in the pupillary portion which was directly exposed. This indicated that heat conduction from the iris and direct absorption by the lens itself had both produced cataracts. Exposures to lower power levels over longer periods of time also produced cataracts reliably. Table I shows cataract development as a function of total energy and exposure duration.

The development of threshold cataracts behind the iris seems to correlate well with the total energy of the exposure rather than with the power levels. The exposures to the iris produced mainly peripheral opacities, whereas every lens exposed to an energy of 100 J or more developed a solid opacity over the entire anterior surface of the lens. There was also evidence for a good reciprocal relation between power and exposure durations for durations up to 23 minutes for a dose of about 100 J. All those exposures produced minimal cataracts. Exposures at still lower power levels have not produced frank cataracts, possibly because the longer duration exposures required to achieve the 100 J dose seemingly required for such an effect were, for technical reasons, not available.

Data from other sources (Wolbarsht, 1980) showed that exposure to broad band infrared radiation (from a Raychem IR 500 Infrared Hand Tool) for 21 hours did not produce any noticeable increase in the cloudiness of the lens. Also, this type of exposure did not result in any changes during an additional two months period after the exposure. However, as explained, there were some shifts in the concentrations of the various crystallins.

B. In Vitro

Experiments reported elsewhere (Wolbarsht, 1980) showed that isolated rabbit lenses incubated at 37°C showed cataract formation when simultaneously exposed to broadband infrared radiation.
No incubated lenses were exposed to laser radiation during the incubation period due to technical difficulties. However, such experiments should be a part of any future program.

II. Analysis Of Exposed Lenses

Following visual examination with a slit lamp the rabbit lenses were removed. Other lenses from calves, rats as well as rabbits were extracted before exposure and placed in an incubating medium for *in vitro* exposure. All lenses were analyzed with the results as described in subsequent sections.

A. Electron Probe Microanalysis

Electron probe X-ray microanalysis of biological structures has been attempted by several workers (Coleman and Terepka, 1972a, 1972b; Galle, 1966; Ingram *et al.*, 1976; Lehrer and Berkeley, 1972; Mizuhira *et*

Table I

Cataract Formation at Various Power Levels and Exposure Times for Laser Exposure to *In Vivo* Rabbit Lenses

Watts	Seconds	Joules	Results
0.1	1050	105	Cataract
0.16	3600	576	Cataract
0.61	180	109.8	Cataract
0.94	30	28.2	Very Slightly Cloudy
0.94	60	56.4	Cloudy
0.94	180	169.2	Cataract
1.10	60	66.0	Cloudy
1.10	180	198.0	Cataract
1.30	60	78.0	Cloudy
1.30	120	156.0	Cataract
1.50	25	37.5	Cloudy
1.50	60	90.0	Cloudy
1.50	90	135.0	Cataract
1.75	50	87.5	Cloudy
1.75	90	157.5	Cataract
2.00	25	50.0	Cloudy
2.00	50	100.0	Cataract

al., 1972; Spear et al., 1971; and Tousimis, 1963a, 1963b, 1969a, 1969b, 1969c, 1970). These investigations were mainly concerned with analysis of minerals in hard and soft tissues, such as calcium in bone and the precipitates of excessive amounts of ions in pathological tissues. Although the method, as yet, has not been standardized in any part of the eye, we attempted to localize, at least, the elements sulfur, copper, and nickel in our first experiments. The experiments on control lenses with galactose cataracts in rats gave inconclusive results as back-scattered electrons from the gold grids interfered with the desired signal. For this reason, future experiments designed to locate sulfur, copper and nickel should employ beryllium grids. However, calcium analysis gave reproducible and reliable results, even though, as yet, the changes in calcium levels are too inconclusive to correlate with other changes in the lens or with cataractogenesis.

B. Amino Acid Levels of In Vivo Laser Exposures

An analysis of the cataractous portion of the lens behind the iris showed no marked changes in the amino acid concentrations following laser exposures. The overall pattern of the amino acid distributions appeared similar to that in the corresponding portion of a normal lens. A record of an amino acid analysis is shown in Table II to indicate the normal levels and typical changes after laser exposure. A comparison experiment with 60 sec exposures at a 1 W power level in a 3 mm laser beam produced cataracts in the portion of the lens irradiated directly. An analysis of the cataractous portion of the lens showed no changes in the majority of amino acids. Cystine, ornothine, and tryptophan were not found at significant levels. Methionine was reduced by a factor of 4 as compared to the normal lens, while the arginine and histidine levels increased by almost an order of magnitude. In the non-cataractous portions of the lens, and in the lens from the companion eye, there were also changes in histidine and arginine. Histidine increased by a factor of 4 in both the nucleus and cortex, while arginine increased by a factor of 2 in the cortex, but remained at approximately the same level in the nucleus. Although the power levels of those laser exposures were quite high, the changes in the lenses may be important features to examine following chronic exposure at lower levels. Although some changes in the amino acid levels were detected, it seems unlikely that these were significant enough to initiate cataractogenesis. As in other exposures the laser beam was filtered to avoid contamination from visible or UV pump light. As compared with gel electrophoresis, the amino acid analysis was not as reliable in detecting the earlier stages of cataractogenesis. On the basis of these experiments together with the gel electrophoresis data, we have inferred that the chief mechanism of infrared cataract formation is most likely protein denaturation, rather than oxidation or some other specific chemical reaction.

C. Electrophoresis of Incubated Lenses

1. Calf Lenses

a. Whole Lens Homogenates.

The initial electrophoresis experiments were conducted on incubated

Table II

Amino Acid Analysis of Normal and Laser Exposed Rabbit Lenses

	Control		CW neodymium laser, 1.06 μm 1.0 W exposure level, 3 mm beam*				
			right eye (beam impinged on iris)		left eye (beam did not touch iris)		
	cortex	nucleus	cortex	nucleus	cortex	nucleus	opaque portion
Alanine	5.1	4.8	4.9	4.9	4.3	4.4	4.8
Valine	5.9	5.5	6.0	6.1	6.0	6.1	5.6
Glycine	9.6	9.3	9.3	9.1	8.7	8.7	8.4
Isoleucine	3.9	4.0	4.1	4.6	4.4	4.7	3.7
Leucine	8.3	9.0	8.8	8.8	8.2	8.7	8.1
Proline	6.8	6.3	6.9	6.9	6.6	6.2	6.4
Threonine	3.4	2.8	3.5	3.5	3.4	3.1	3.2
Serine	9.6	9.8	9.9	10.4	9.8	10.1	9.0
Methionine	2.5	1.9	2.4	2.5	2.0	2.0	0.6
Phenylalanine	6.1	5.9	6.1	5.9	5.6	5.9	5.8
Aspartic acid	10.1	10.1	10.1	9.8	9.0	9.6	9.0
Glutamic acid	15.7	14.8	14.8	14.3	13.7	13.8	13.9
Tyrosine	4.7	6.1	5.3	5.0	5.0	6.0	4.7
Lysine	4.7	4.1	4.9	4.5	4.6	4.4	4.4
Histidine	trace	0.8	trace	0.81	2.7	0.7	5.3
Arginine	2.7	3.7	?.4	2.5	5.3	4.8	6.5
Cystine	0.8	1.2	0.9	0.4	0.6		

*Two exposures to each eye, one day apart: #1, 63 seconds; #2, 60 seconds

normal calf lens homogenates as the lens proteins have been well characterized by previous workers (Zigler, 1975). The heated samples showed changes in the thin gel isoelectric focused electrophoresis patterns after as little as one-half hour at 45°. There were no significant precipitates, although all solutions became somewhat cloudy with longer incubation.

The incubation temperatures ranged from 37° to 45° C. Isoelectric focused gel analysis of the lenses incubated one-half hour at 45° showed a definite loss in the lowest points of the α crystallin components. This trend continued with increased duration of the incubation until after 24 hours at 40° to 45° C all of the α crystallin had disappeared. Similar changes occurred at lower temperatures (even at 37° C) but at a reduced rate. No significant precipitates occurred in any samples, but all appeared more opalescent or cloudy with the longer treatments. The loss of α crystallin from the isoelectric focusing pattern was not due to decreased solubility, but apparently resulted from aggregation of the larger α crystalline components to even higher molecular weight particles which could not enter the gel. Investigation of the size of distribution of the calf lens homogenate after 24 hours at 45° on a Biogel A5M column indicated no change in the β_H and β_L crystallins. However, the α crystallin peak shifted to the void volume of the column, indicating an increased molecular size. In addition, a very high molecular weight fraction (greater than 5,000,000) appeared which contained predominately α crystallin material. This is shown in Figure 1.

Gel electrophoresis of the separated fractions as described below made further characterization possible, although no changes in the individual patterns of the incubated lenses were seen.

Control lenses held at 4° C or at room temperature (20° to 25° C) for 24 hours had the same electrophoresis patterns as freshly prepared samples measured the same day.

b. Isolated Lens Crystalline Proteins.

The purified, isolated crystallins were incubated to pinpoint possible changes in the homogenized or whole lenses. Control solutions of all proteins left at room temperature for 24 hours were unchanged. The α, β_H, β_L, and γ crystallins were analyzed separately by gel electrophoresis after incubation at temperatures ranging from 40° to 45° C for 22 hours.

The isolated lens crystallins were unchanged as compared with fresh samples for periods of 24 hours at room temperature. However, some changes were found after incubation for 22 hours. For example, the α crystallins showed changes quite comparable to those in the whole calf lens homogenate. Column chromatography indicated that the isolated α crystallin had shifted to a slightly higher molecular weight. The very high molecular weight soluble aggregate (greater than 5,000,000) was much less than that seen in the whole lens homogenate, indicating that traces of the β or γ crystallins may assist in the formation of this very large, but soluble aggregate. Calcium (0.01 M $CaCl_2$) had no effect on α crystallin aggregation in this system.

Some β_H crystallins formed a heavy flocculant precipitate at temperatures

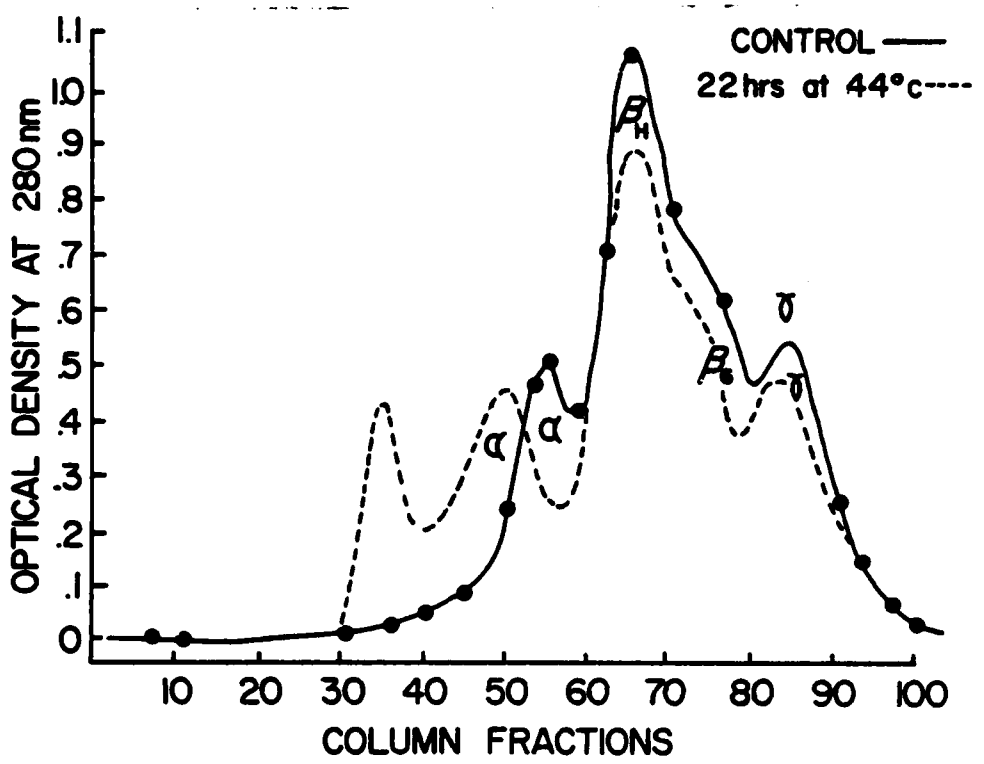

Figure 1

Analysis of calf lens homogenates on a Bio Gel A5M column showing the optical density at 280 nm following incubation for 22 hours at 44°C in 0.85 saline. The only change was in the α crystallin moiety which shifted into a larger size (lower fraction number), and appears in the void volume of the column for the control homogenate. Control lenses held at 4°C or at room temperature (20 to 25° C) for the same length of time (24 hours) were not changed detectably from fresh samples tested immediately.

above 40° C in less than one hour. Electrophoresis in SDS solution of the precipitated material showed a higher molecular weight band in the insoluble fraction not present before heating. This component did not seem to result from disulfide formation, or at least not entirely so, since the use of sulfhydryl sulfide agents did not dissociate the component to smaller units, or even prevent or retard precipitation in the original protein solution. Possibly, nondisulfide covalent bond may be involved.

The isoelectric thin layer gel electrophoresis focusing results were difficult to interpret because of the overlap of the many bands. For that reason samples of both the precipitate and the soluble material were disassociated in SDS solution.

Electrophoresis of samples dissociated with SDS showed marked differences in the insoluble fraction. The heated β_H crystallin contained a high molecular weight band which was not present before heating. This component does not seem to result from the disulfate formation or at least not completely so, as the sulfhydryl reagents did not disassociate any of these components into smaller units. Thus, non-disulfide covalent bonds may be involved in the aggregation. In addition, the inclusion of sulfhydryl reagents into samples of the original protein solution did not prevent or retard precipitation during incubation.

The investigation of heated β_H crystallin by thin layer gel filtration on Sephadex G-200 indicates some breakdown of quartenary structure. Heat treatment for one and one-half hours at 45° C seems to reduce most of the soluble material to the dimer state as compared with the 5-7 peptide chain native state.

β_L crystallin was precipitated during the heat treatment but not as heavily as the β_H. Although the β_L precipitation proceeded more slowly than the β_H, it did occur at lower temperatures. In its native state β_L is a dimer and it does not contain the two relatively high molecular weight polypeptides that probably accounted for the majority of the β_H precipitate. As with β_H the insoluble portion of the β_L contained high molecular weight aggregates and was characterized by particular β_L polypeptides.

The isolated γ crystallin did not appear to be affected by the elevated temperatures for the durations used in our trials, possibly due to the fact that the purified γ crystalline preparations did not have the minor γ component of the lens homogenate that was reduced by heating for long durations.

Mixtures of crystallins were incubated in order to determine whether any of the individual crystallins were stabilized by the presence of others. Adding β_L to β_H did not affect the onset of precipitation of β_H. However, adding α and γ crystallins in amounts equal to the β_H crystallin prevented the β_H precipitation for up to 24 hours, whereas under the same conditions the β_H alone would precipitate within one hour. This type of stabilization is similar to that seen in the incubation of whole lens homogenates in which there is no β_H crystallin precipitation within the first 24 hours. Possibly the α crystallin present in the whole lens homogenates is the stabilizing agent.

c. pH Dependence

Buffering the lens protein solution to a lower pH seems to potentiate any effects from heating, i.e. the effects from heatings show a pH dependency.

The calf lens homogenates showed a decrease in the α crystalline fraction at pH 5.0 following incubation for 22 hours, but no detectable changes were found at the higher pHs of 7.0 and 8.0. The change at pH 5.0 was similar to that seen in the calf lens homogenate in unbuffered saline. On the other hand, thin layer isoelectric focused gel electrophoresis did not show any changes with pH, nor did pH changes cause any observable precipitates in any of the samples.

The isolated α crystallin response to heating changed with pH just as the α crystallin in the whole homogenate.

The isolated γ crystalline solution remained completely clear during the entire incubation period and the electrophoresis pattern for pH 8.0 had the lowest isoelectric point missing completely, although the other pH values were unchanged from controls.

β_H solutions became cloudy rather quickly at the lower pHs and precipitated within about 4 hours. The same protein in unbuffered saline precipitated more slowly, and the precipitate was not as heavy. However, the nature of the precipitation appeared to be identical to that found in unbuffered saline solution. After 22 hours, all β_H samples contained some precipitate, although at pH 6.5 and 7.0 had the least.

The β_L solutions were similar to β_H in that precipitation was first observed in the pH 5.5 and 6.0 samples. After the total 22 hour period, all samples contained precipitates but with only slight amounts in pH 6.0, 6.5 and 7.0.

These results suggest that some of the changes in the homogenized calf lens incubated in saline are in part due to the low pH. Consequently in future studies on lens homogenates and isolated lens proteins as well as whole lenses, the solutions should be buffered to near neutral. Our trial with neutral buffers did not show any changes in the α crystallins while the γ crystallins showed effects not observed at lower pHs. The β_H and β_L crystallins in neutral solutions show much the same changes but reduced in magnitude as in unbuffered saline.

2. Rat Lenses

The chemical changes in the incubated lenses were studied before any actual opacities began to form. Lenses from older rats (10 weeks) appeared less susceptible to heat stress than the younger lenses (4-6 weeks). Younger lenses therefore were used because of the ease of cataract formation. The 4-6 week rat lens maintained its transparency for up to 13 days at 34.5° C, 8 days at 37.5° C, 7 days at 38.5° C, and only 3 days at 40.0° C. In each case, only completely clear lenses were analyzed.

Figure 2 shows the isoelectric focusing patterns of thin-layer gels (pH 5-8) for samples of the lens proteins incubated at various temperatures.

The obvious loss of the band in the 40° C sample suggests that the threshold temperature for causing changes in the lens proteins is between 38.5° and 40° C. A similar result is seen in a gel made up at a broader pH (pH 3.5-10). Again there is the loss of a band which is thought to represent one of the β crystallin.

3. Rabbit Lenses

A control rabbit lens shows 8 distinct bands on the SDS gel (see left hand gel in Figure 3). Incubation at 40° C for 3 days gave no changes from the control. However, after 4 days, band 3 was reduced in concentration. Band 3 corresponds to a β crystalline component of the purified lens proteins which was reduced in concentration in similar fashion but much slower than that from the IR ray gun lamp exposed rabbit lenses.

D. In Vivo Laser Exposures

Laser exposures at levels above 50 J usually caused some degree of cloudiness in the lens. The energy levels below 90 J (1.50 W, 60 sec) produced primarily peripheral opacities beginning where the ciliary muscles attach to the lens capsule. Every lens exposed to energy levels of 100 J (2.00 W, 50 sec, for instance), or more, developed a solid opacity over the entire anterior surface.

The analysis of various parts of the exposed and unexposed lenses by SDS Gel electrophoresis following typical exposures are shown in Figure 4.

Where a visible opacity was present (Brown, #1, and Brown, #2,), the opacified portion of the lens was studied separately. In these opaque portions, nearly all soluble crystallins (α, β_H, β_L, and γ) had disappeared. This implied that the opacity following the laser exposure resulted from the formation of high molecular weight albuminoids, a portion of which, at least, was derived from these soluble crystallins. The clear portions of all exposed lenses that developed opacities retained their soluble crystallins. However in the clear portion the β crystallins showed a significant change towards a lower mobility, suggesting that agglutination of the β fractions is the initial step in the formation of the insoluble albuminoids. The portions of exposed lenses that did not develop an opacity showed a similar trend for the β crystallins. From this it can be concluded that the IR effect on the lens is vibrational in nature with the β crystallin protein unfolding slightly to expose charged amino acid or sulfhydryl groups.

Electrophoretic analyses of typical laser exposures are shown in Figures 5, 6 and 3 in which the separated protein bands of exposed and control lenses are separated by electrophoresis of various types, isoelectric focusing using urea and non-urea gels, and SDS gels.

Figure 2. Thin-layer acrylamide gel (pH5-8) showing protein bands from rat lens incubated at different temperatures. Arrow indicates missing band assumed to represent β - crystallin.

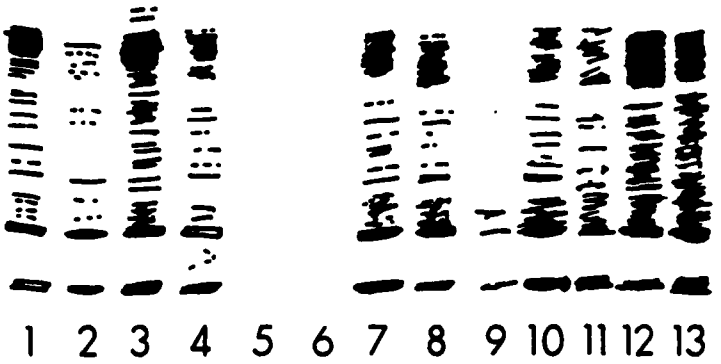

Figure 4. Thin layer gel electrophoresis of rabbit lenses following laser exposure. #1, #2, and #3 are the controls. #1 is the whole lens; #2 is the nucleus; #3 is the cortex. #5, #6, and #7 are brown rabbits exposed in each eye for 7 seconds to 10 W. #4 is a portion from the dilated eye; #6 is a portion of the opacity formed; #5 and #6 are portions of the opacity from each eye; #7 is the rest of the lens from the undilated eye. #8, #9, #10, and #11 are from rabbits exposed to 2 W for 40 seconds. #8 and #9 are from the undilated eye; #10 and #11 are from the dilated eye. #11 is from the opaque portion of the dilated eye while #9 was slightly cloudy. #12 and #13 are from brown rabbit exposed to 2 W for 20 seconds. #12 is the whole lens from the nondilated eye; #13 is the whole lens from the dilated eye. See text for explanation of the patterns.

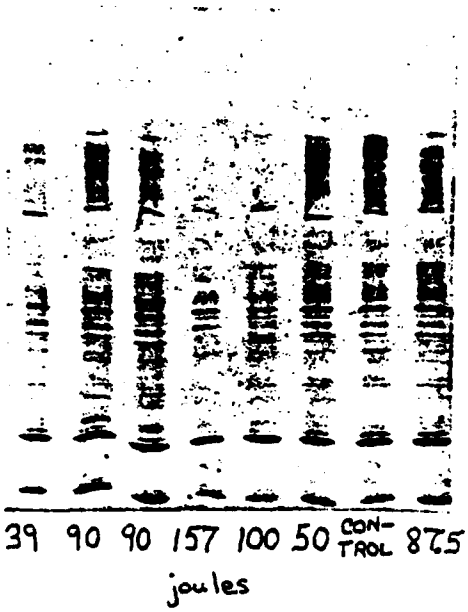

Figure 5. Thin-layer acrylamide gel showing laser exposed rabbit lens protein.

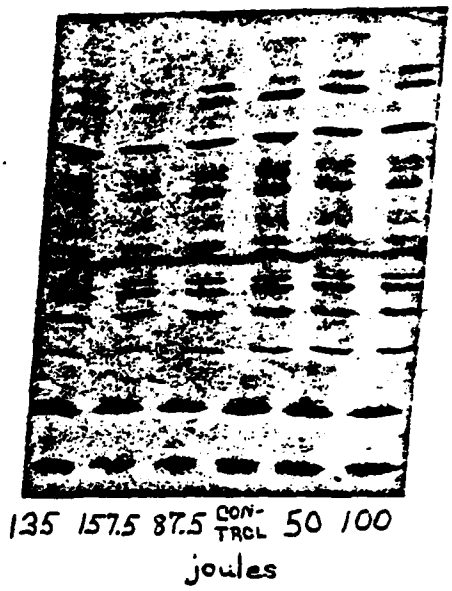

Figure 6. Thin-layer urea gel showing laser exposed rabbit lens protein.

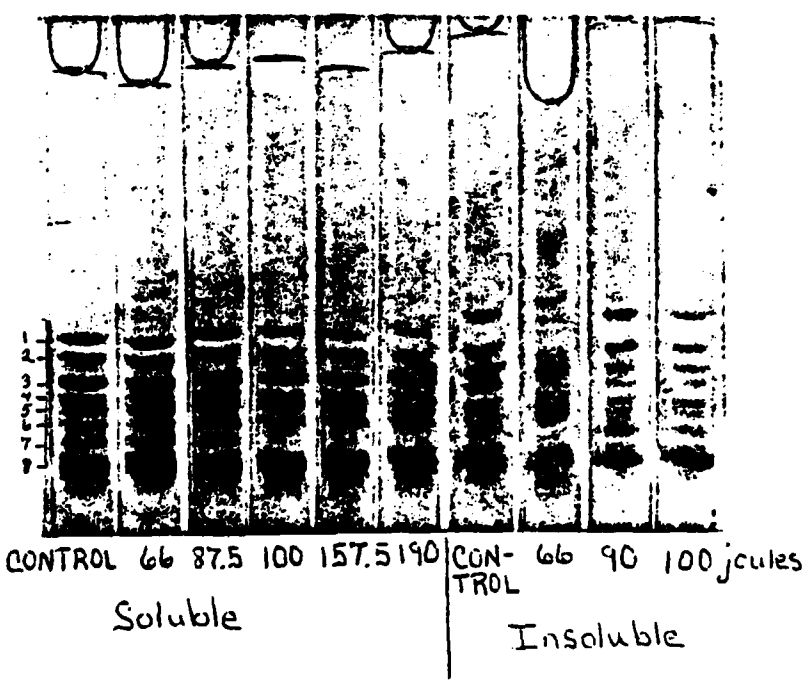

Figure 3. SDS gels showing laser exposed rabbit lens protein.

Figure 5 (a thin-layer acrylamide gel, pH 3.5-10) shows a decrease in the α crystallin in the samples irradiated at higher energy levels, i.e. 100 J (2.00 W, 50 sec), 157.5 J (1.75 W, 90 sec). Studies using lens homogenates showed the same loss of some α crystallin bands and the creation of a high molecular weight crystallin detected by column chromatography. This compound may be similar to that which the high-molecular weight protein fraction with molecular weight above 10,000 Daltons in calf lens which turned out to be composed of α crystallin polypeptides (Spector et al., 1971a, b).

Figure 6, analysis of the samples using a 6 M urea gel, exhibits a loss of a distinct band in the high energy exposed samples, i.e. 135.0 J (1.50 W, 90 sec), 157.5 J (1.75 W, 90 sec), 87.5 J (1.75 W, 50 sec), 100 J (2.00 W, 50 sec). Unfortunately, a definite identification of this band was not made. In Figure 3 (SDS acrylamide gel), the gradual loss of band 7 in the soluble fraction with increasing energy level of laser exposure is comparable with the gradual appearance of band 7 in the insoluble fraction. Band 7 corresponds to the band produced when purified α crystallin is run on SDS gels. This is evidence of a transition of soluble α crystallin to an insoluble protein form.

Throughout the experiments using rat lens homogenates and whole lens organ cultures incubated at a temperature of 40°, as well as in rabbit lenses exposed to a CW neodymium laser (1060nm), analysis by thin-layer isoelectric focusing has shown a decrease in the α crystallin fraction of the soluble protein. In the clear portions of lenses in which exposure from a CW neodymium laser produced an opacity, the β_H crystallin fraction showed a markedly decreased mobility suggesting that in the urea thin layer gel (Figure 6) there are indications that a urea soluble component is dropping out in the higher irradiated samples. It could be that if this urea-soluble compound is formed from HM crystallin, the stress of the high energy impingement could cause the urea-soluble compound to break down. This would decrease its concentration as seen in the 135 J (1.50 W, 90 sec), 157.5 J (1.75 W, 90 sec), 87.5 J (1.75 W, 50 sec) and 100 J (2.00 W, 50 sec) samples in Figure 3 and possibly account for part of the increase in the HM crystallin. The loss of band 7 (Figure 6) in the soluble fraction and the increase of band 7 in the insoluble fraction is evidence of a transition of protein from the soluble to the insoluble form as a result of laser exposure. From this the action of laser exposure can be considered a premature or accelerated aging process. For example, an early study by Dische et al. (1956) shows that in the aging rat lens the crystallin fraction decreases directly as the insoluble albuminoid protein fraction increases.

Exposures at a power level of 0.223 W very quickly produced obvious thermal damage, demonstrating that use of such high levels would cause so much damage as to swamp out any suitable biochemical changes of a precursor nature. At 0.223 W, exposure for 2 min (28 J) produces an opacity on the edge of the cornea and indications of a lenticular opacity beginning underneath the corneal opacity. Exposure for 5 min at this same power level evidently heated the speculum enough to burn the eyelids. In both cases, the SDS gel analysis produced no conclusive results, as all bands were affected.

At 0.16 W, an hour of laser exposure produced an almost complete lenticular surface opacity both anterior and posterior. Only a central disc on the posterior side remained clear. This time power combination represents an exposure of 576 J. At this level, a great deal of the soluble protein is converted into insoluble protein and does not produce band patterns on the SDS gel. Such an energy level is still too destructive to the lens proteins to enable identification of the subtle changes sought.

Laser exposure at 0.1 W for 17.5 min produced a ring-shaped opacity on one edge of the cornea which corresponded to a lenticular surface opacity directly underneath. This time/power combination represents an exposure level of 105 J (0.1 W, 1050 sec). The SDS gel electrophoresis shows band 7 (presumably an α crystallin) decreasing in concentration at this energy level. This observation is identical to results at the same energy levels reported previously with higher wattage, but shorter (and fractionated) exposure times (2 W, 50 sec). This indicates that the dose is the important parameter, and that reciprocity between power and time holds over more than 1 order of magnitude.

Additional exposures (from 15 to 23 min duration) all showed that at about 100 J there was a marked decrease in the α crystallin protein (band 7 on the electrophoresis gel). This obviously dose related type of relationship argues very heavily against any type of thermal model for infrared cataractogenesis from chronic exposure. The exact mechanism by which the α crystallin is affected is at the present unknown. However, exposure for longer periods of time and lower levels may fractionate the lens response. The change may be confined to the α crystallin, or alternatively, studies on α crystallin alone may show how the infrared affects this molecule. However, it should be kept in mind that extracted lens proteins do not act the same as they do in vivo or even in vitro. Our studies have also indicated that the intact lens has some protective features for α crystallin. Even mixtures with other types of lens proteins appear to prevent infrared degradation of α crystallin. That is, isolated α crystallin is degraded much more rapidly than is the α crystallin component of mixtures with other purified lens proteins.

CONCLUSIONS AND SUGGESTIONS FOR THE FUTURE

We found specific changes in the lens proteins following IR exposures both in vitro and in vivo. These changes seem to be an acceleration of the normal aging process. However, at the present time insufficient data has been accumulated to make a definite statement about safe ocular levels for exposure to laser sources in the IR. The methology used to detect minimal changes in the lens proteins immediatedly following IR radiation has been sufficiently tested to provide quantitative and reproduceable ways of detecting early changes in α crystallins. These changes in the γ crystallins have been linked with cataract development following higher power level exposures by 1064 nm radiation from a Nd laser. It is suggested that an extensive program using these techniques could allow collection of sufficient data to give a quantitative formulation for non-hazardous IR laser exposure levels. However, the possibility still remains that exposure to one region of the near infrared may induce cataract formation by a mechanism which differs from the producing cataracts following exposure from another region.

Another point that should be examined in the future is the indication that in some forms of cataract the cortex of the lens decreases in size. B. Philipson (personal communication) has suggested that normally the nucleus grows at the expense of the cortex, and that the decreased rate of fiber repair and protein synthesis in an enlarged nucleus contributes to the development of pathological degradation in the cortex following any environmental trauma.

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