We conducted reproducibility studies on an in vivo objective method of documenting cataracts: Scheimpflug photography using the Topcon SL 45 camera. Normal and cataractous lenses were photographed and the photographs digitized and analyzed using a Perkin Elmer microdensitometer attached to a PDP 11 and Vax 11/780 computer. Linear densitometry was performed through the center of the lens. We found very good reproducibility. The intraclass correlation for the mean densities in the nucleus was 0.95: that is, intra- and interobserver variability accounted for only 5% of the overall variability. For the anterior cortex, intraclass correlation was 0.88 and for posterior cortex it was 0.84. This method may be useful, within limits, in future clinical trials of anticataract medications. Invest Ophthalmol Vis Sci 28:1707-1710, 1987

With the advent of drugs that have been shown to prevent or reverse cataracts in animal experiments\(^1\)\(^2\) there is presently a need for an objective and reproducible method of documenting and monitoring cataract formation, progression and regression in patients.\(^3\) Methods currently available are either subjective or objective. Subjective methods such as visual acuity testing, glare testing, contrast sensitivity testing and slit lamp biomicroscopy are susceptible to a wide variety of factors and variables, making them difficult to standardize. This renders them unsatisfactory for use alone for clinical trials to assess drugs that slow down or prevent the development of cataracts.\(^3\)\(^4\) Objective methods such as slit lamp photography, Topcon SL 45 and Zeiss Scheimpflug photography, Kawara Retrolillumination photography, scanning laser slit lamp photography, quasi-elastic laser light scattering spectroscopy, magnetic resonance imaging and others allow for standardization but need to be tested for reproducibility and adequacy of correlation with clinical findings.\(^3\)\(^5\) These subjective and objective methods were discussed more extensively in a recent review.\(^3\)

This paper deals with an objective method: Scheimpflug photography. We conducted a study at the National Eye Institute to determine the reproducibility of Scheimpflug photography using the Topcon SL 45 Scheimpflug camera quantitatively for studying different types of cataracts. Age-related changes are not dealt with in this paper.

**Materials and Methods.** The Topcon (Tokyo Optical Co., Ltd., Tokyo, Japan) SL 45 modified slit lamp camera was developed according to the Scheimpflug principle. This principle states that the image of an obliquely positioned object is formed such that the planes of the object, image and objective intersect.\(^6\) This allows for the sagittal image of the anterior segment of the eye to be photographed such that it is in focus from the anterior surface of the cornea to the posterior surface of the lens. The camera can be rotated 180° along the visual axis, so the entire anterior segment could be photographed. In addition, it has two important features: an internal standard, a gray scale of five steps, which is incorporated in the photograph allowing for standardization of film image; and a photoacoustical fixation device\(^7\) allowing for precise alignment of the patient’s eye (in the presence of reasonably good visual acuity).

Informed consent was obtained of all patients included in this study. Twenty-eight patients (48 eyes) were dilated maximally using 10% phenylephrine and 1% tropicamide ophthalmic solutions. There were 14 normal lenses, 10 cortical, 8 posterior subcapsular (psc), 11 nuclear, 2 combined cortical and PSC and 3 combined nuclear and PSC cataracts. Two of the authors (M.D. & P.E.) each photographed right and left eyes at a slit thickness of 0.08 mm, axis at 90°, and repeated this after a brief rest period. The sequence of eyes photographed, as well as which observer photographed first or second, was selected in a random fashion. The patients’ fixation was controlled by a flashing green fixation light, as well as by the aforementioned built-in photo-cell acoustical device. The images were photographed on Kodak Tri-X film from a single batch of film emulsion (same lot number), which was carefully developed in a standardized fashion by the NEI Clinical Branch Photography Section using a 1:1 concentration of Kodak D-76 developer.

The 35 mm film images were digitized using a Perkin-Elmer (Garden Grove, CA) 1010MG microden-
Fig. 1. Digitized image of the Scheimpflug photograph of the anterior segment of the eye. This shows the slit through the center of the gray scale and across the cataract and cornea which was analyzed by microdensitometry. Under the image is the resultant graph of the vertical average of the density of the pixel values in the central horizontal area analyzed. Note that the major regions of the lens (nucleus, anterior cortex and posterior cortex) are delineated by the computer program.

Figures 2-4 show the scattergrams of the means of the pixel density values in the different regions of the lenses examined. One image was arbitrarily chosen for each of the 48 lenses.

Mean density values in the nucleus were calculated for different categories of lenses, grouped as nuclear cataracts, non-nuclear (cortical or psc) and normal lenses (Fig. 2). These values were 0.83 OD for nuclear cataracts, 0.64 OD for non-nuclear cataracts and 0.56 for normal lenses. These values demonstrated statistically significant heterogeneity (P < 0.001) between classes, although there was overlap of densities in individual lenses. Similar results were obtained when mean, median or 90th percentile optical density (OD) units were analysed.

Mean density values in the anterior cortical area (Fig. 3) showed significant heterogeneity between normal lenses and cataractous lenses (P = 0.01), but there was no difference between cortical cataracts and non-cortical cataracts (normal = 0.68 OD, cortical = 0.79 OD and non-cortical = 0.79 OD).

Mean densities in the posterior cortical region (Fig. 4) also showed significant heterogeneity (P < 0.001) between the different types of lenses. In addition, there was less overlap between the individual lenses across the categories than in other zones. These values were, for psc cataracts 0.63 OD, for non-psc cataracts 0.41 OD and for normal lenses 0.34 OD.

Discussion. The Topcon SL-45 Scheimpflug camera provides an objective and reproducible method of

* Software is freely available to interested parties.
evaluating lens opacities by recording an image of the lens which can be analyzed by linear densitometry. The results demonstrate that there was far less variability in the nuclear zone, ie 5%, compared with 12% for the anterior cortex and 16% for the posterior cortex. This may limit its usefulness in clinical trials to assessing changes primarily in the nucleus.

The lenses studied here were chosen arbitrarily to represent normals and three classes of cataract; selecting a different sample could affect the results. In particular, we have used the intraclass correlation as a measure of reproducibility, which indicates what proportion of total variability (among all four images of the same eye) was due to differences between lenses (as opposed to differences between repeat images of the same lens). This measure depends on the heterogeneity of the group of lenses in the study.

This method also allows for an automated and objective method of detecting differences in opacity. Although there was significant heterogeneity between classes of cataracts on average, particularly for nuclear and posterior subcapsular cataracts, there was overlap of individual lenses across categories, which could adversely affect attempts at automated classification. In particular, cortical cataracts were difficult to distinguish from non-cortical cataracts. However, it is possible that this approach would be useful in following changes in a particular lens over time, but we have not yet investigated this question. Age-related changes in the cataractous and non- cataractous eyes do not interfere with the reproducibility test performed here and are not addressed in this paper.

In addition, this method does not allow for a complete documentation of all areas of the lens, particularly lens cortical areas, so that one needs to photograph many angles in order to get representative areas around the lens. Photographing more meridians (for example, 180°, 135° and 45°) may help improve reproducibility for the cortical regions. With this, one will gather more information on changes occurring in the cortex. However, this may also introduce some error which may occur from the repositioning of the camera or from the bleaching of the patient's photoreceptors which may affect fixation. Since cortical changes are heterogeneous (dots and clefts), many cortical opacities will still be missed. Other authors have also noted this problem and believe that this technique in its present form may be too inefficient to be clinically useful for cortical opacities. However, as long as one is aware of the limits of this instrument, it can still be useful for studying cortical opacities.

Another problem encountered is the difficulty of correlating the images with what we see when examining the patient at the slit lamp biomicroscope (validity). However, it may be possible to overcome these difficulties if three-dimensional reconstruction of the images can be accomplished, in such a way that one can scan the cortical areas in the entire lens, and
correlate this with the three-dimensional image one forms in one's mind when examining a patient on a slit lamp. We are currently pursuing this possibility using the newly introduced Zeiss Scheimpflug video camera. In addition, different image analysis and densitometric methods need to be developed to process the images generated by this system.

**Key words:** cataracts, Scheimpflug photography, image analysis, reproducibility

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From the *Clinical Branch and Biometry and Epidemiology Program, National Eye Institute and the Image Analysis Section, Division of Computer Research and Technology, National Institutes of Health, Bethesda, Maryland.* Presently with the Clinical Trials Branch, National Cancer Institute, National Institutes of Health. Presented at the Association for Research in Vision and Ophthalmology Meeting, 1986, Sarasota Florida. Submitted for publication: October 28, 1986. Reprint requests: Dr. Manuel B. Datiles, CB-NEI, NIH, Building 10, Room 10N226, Bethesda, MD 20892.

**References**


**Cysteamine Induces Cataracts in Newborn Rats**

Toan Truong,* Stephen M. Sagar,* William J. Millard,† and Jennifer L. Shaw‡

Daily subcutaneous injections of cysteamine (CSH) administered to neonatal rats for 6 to 11 days cause dense bilateral cataracts that are evident at eye opening and that are permanent. CSH has been previously shown to induce protein cross-linking by the formation of abnormal disulfide bonds. It is hypothesized that the same mechanism underlies its cataractogenic effect; as such, CSH may be a useful agent in the study of models of senile cataract formation in humans. Invest Ophthalmol Vis Sci 28:1710–1713, 1987

Cysteamine (CSH, 2-mercaptoethylamine) is a sulfhydryl agent which catalyzes the rearrangement of disulfide bonds in vivo.1,2 In the process of studying the neuroendocrine effects of this agent, we noted that neonatal rats given systemic CSH in high doses developed bilateral cataracts. Since there is evidence that abnormal disulfide bond formation is involved in senile cataract formation,3–6 we systematically studied the effects of CSH on the lens.

**Materials and Methods.** Animals: Timed pregnant Long-Evans and Sprague-Dawley rats (Charles River Laboratories, Medford, MA) were maintained on a 12 hr, 12 hr light/dark schedule with free access to food and water. During the first day of life, pups were randomly reassigned to mothers and litter sizes were equalized. Injections were made subcutaneously in the back of the neck between 2:00 PM and 5:00 PM. The experimental methods conform to the principles of the ARVO Resolution on the Use of Animals in Research.

**Chemicals:** Doses of cysteamine hydrochloride (Sigma Chemical Company, St. Louis, MO) and ethanolamine hydrochloride (Aldrich Chemical Company, Milwaukee, WI) are expressed as mg of the hydrochloride salts. Stock solutions of CSH, 20 mg/ml, and an equimolar concentration of ethanolamine, 17 mg/ml, were prepared on the day of administration and neutralized with NaOH.
Injection schedule: For Experiment #1, performed with neonatal Long-Evans rats, Group A received an increasing dose of CSH as follows: 100 mg/kg on days 1 and 2 of life, 150 mg/kg on days 3 and 4, 200 mg/kg on days 5 and 6, and 250 mg/kg on days 7 and 8. Group B received a constant dose of 200 mg/kg for the first 11 days of life. The controls received the same molar dose of ethanolamine as the CSH dose of Group A.

Experiment #2 was performed with albino Sprague-Dawley rats. One group received CSH at a constant dose of 200 mg/kg per day for the first 6 days of life. A second experimental group received CSH 200 mg/kg per day on days 10–16 of life. The corresponding control groups received an equimolar dose of ethanolamine.

Examination of the lens: The pigmented Long-Evans rats were sacrificed on days 18–36 of life, and the cataracts were examined with a photomicroscope. The lenses were immersed in saline at room temperature and photographed immediately using incident lighting. It is necessary to photograph the lens immediately, as they will cloud if left in room temperature saline for more than about 20 min. This generalized clouding of the lens could be readily distinguished from the CSH-induced cataracts. The lens of the Sprague-Dawley rats were inspected in vivo.

Results. There was a high mortality among the CSH-treated pups (Tables 1 and 2). Moreover, the CSH-treated rats had delayed growth, eye opening and sexual development. On day 15 of life, only 5 of 15 CSH-treated rats in Group A had completed eye opening, compared to 13 of 14 in the ethanolamine-treated group. The CSH-treated Long-Evans rats had hypopigmented fur over their backs and to a lesser extent over their faces.

Tables 1 and 2 demonstrate that both dosage schedules of CSH reliably produce cataracts in neonatal animals. Animals surviving as long as 4 months had no resolution of the cataracts. If CSH administration is delayed until day 10 of life, cataracts do not result (Table 2, Period 2). CSH administration to adult Long-Evans rats at a dose of 100 mg/kg for 16 days fails to induce cataracts.

Figure 1 demonstrates the appearance of the lens of representative CSH-treated rats. Most cataracts are dense, white, irregular in shape, and nuclear in location (Fig. 1B). A few cataracts were posterior in location, as shown in Figure 1C. Lenses from control animals never had opacities.

Some Long-Evans rats had unusual pigmented strands extending from the iris to the lens (Fig. 1D). The pupils of these rats were irregular in shape.

Discussion. CSH has many biological effects. It inhibits dopamine β-hydroxylase, lowers the concentration of immunoreactive somatostatin in the gastrointestinal tract and central nervous system and depletes immunologically and biologically active prolactin from the serum and anterior pituitary gland (see ref. 7 for review and detailed references). The neuroendocrine activity of CSH is related to the ability of the compound to catalyze the rearrangement of disulfide bonds. CSH has been used clinically to treat acetaminophen poisoning and hereditary nephropathic cystinosis, and it is the most potent radioprotective agent known.

CSH administered at a relatively high dose to neonatal rats daily for 6–10 days reliably produces permanent cataracts. We currently have no information concerning the effects of lower doses, nor do we have biochemical data concerning the mechanism of cataract production. We hypothesize that, in analogy with the neuroendocrine effects of CSH, the cataracts are due to cross-linking of proteins by abnormal disulfide bridges. In the lens, cross-linking of proteins by any means increases their effective molecular weight and produces light scattering and consequent lenticonus opacity. The production of such high molecular weight protein complexes by disulfide bridges has been implicated in the formation of senile cataracts in humans.

Cataracts may be produced in neonatal mice by buthionine sulfoximine and in 9–15-day-old rats

<table>
<thead>
<tr>
<th>Table 1. Cysteamine-induced cataracts in Long-Evans rats</th>
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<tbody>
<tr>
<td><strong>Survivors</strong></td>
</tr>
<tr>
<td>Control</td>
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<tr>
<td>CSH, Group A</td>
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<tr>
<td>CSH, Group B</td>
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Experiment 1: Newborn Long-Evans rats in Group A were injected with an increasing dose of CSH from 100 mg/kg to 250 mg/kg for 8 days, as described in Materials and Methods. Group B received a constant dose of 200 mg/kg for 11 days. The control group received ethanolamine-HCL on a schedule and molar dose corresponding to Group A.

Table 2. Cysteamine-induced cataracts in albino rats

<table>
<thead>
<tr>
<th>Period</th>
<th>Survivors</th>
<th>Percent survival</th>
<th>Percent survivors with cataract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period 1</td>
<td>Controls</td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td>CSH</td>
<td>9</td>
<td>32</td>
<td>100</td>
</tr>
<tr>
<td>Period 2</td>
<td>Controls</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>CSH</td>
<td>37</td>
<td>82</td>
<td>0</td>
</tr>
</tbody>
</table>

Experiment 2: Rats were given a constant dose of CSH, 200 mg/kg, for a 6 day period beginning on either day 1 of life (Period 1) or day 10 of life (Period 2). Control groups received an equimolar dose of ethanolamine-HCL.
with selenite.\textsuperscript{9} Both agents deplete the lens of reduced glutathione and may also lead to the abnormal rearrangement of disulfide bonds. It is also of note that buthionine sulfoximine and selenite, like CSH, are only effective in the immediate postnatal period.

Mechanisms of action of CSH other than a direct disulfide exchange reaction with lens protein are possible. For example, reaction of rabbit lens in vitro with parachloromercuribenzenzene sulphonic acid, which reacts with exposed membrane sulfhydryl groups, leads to excess calcium entry into the lens and opacification.\textsuperscript{10} It is possible, therefore, that CSH acts through a similar mechanism.

Our results add to the evidence that the sulfhydryl exchange may be involved in cataract formation. CSH, buthionine sulfoximine and selenite may all prove to be useful experimental agents in studying this mechanism. These data, in concert with the results of other laboratories,\textsuperscript{3,4,6} suggest a possible relationship of these cataracts in neonatal rodents and senile cataracts in humans.

**Key words:** cataract, cysteamine, disulfide, sulfhydryl, lens

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From the *Neurology Service, VA Medical Center and University of California, San Francisco, California, the ‡Department of Pharmacodynamics, College of Pharmacy, University of Florida, Gainesville, Florida, and the ‡Albany Medical College, Albany, New York. Supported by the National Eye Institute (EY-05721). Submitted for publication: February 11, 1987. Reprint requests: Stephen M. Sagar, MD, Neurology Service (127), VA Medical Center, 4150 Clement Street, San Francisco, CA 94121.

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Marked inflammation of the cornea (keratitis solaris) is often observed after exposure to strong solar radiation, especially in high altitudes and snow-covered terrain. Using the action spectrum and the solar spectrum, the radiant exposure causing keratitis solaris was calculated for a horizontal surface. The parameters selected were altitude, season, ozone content and albedo. The radiant exposure of the eye increases approximately 16-fold, comparing terrain without snow-cover and terrain with snow-cover. Radiant exposures in clinically observed cases of keratitis solaris were calculated to range from 1200 to 5600 Jm⁻². A discussion on these figures with regard to threshold doses shows a significant difference between long-term exposure to solar radiation and short-term exposure to artificial sources.

Data of threshold radiant exposure for keratitis photoelectric after short-term exposure to artificial sources are given by Pitts¹,² as amounting to 40 Jm⁻² relative to 270 nm. In the present paper it is shown that this value is hardly applicable for solar radiant exposure. In order to examine the threshold radiant exposure for keratitis solaris, the radiant exposure for individuals with keratitis solaris was estimated by model calculations.

**Materials and Methods.** The model calculations are based on the action spectrum of keratitis phototn-electrica,³ which is normalized to 100% at 270 nm (Fig. 1a), and on the spectrum of global radiation on a horizontal surface given by Bener⁴ (Fig. 1b), whose data are based on extensive spectral measurements. The parameters selected were altitude, ozone content and solar elevation. The calculation results in a keratitis-effective solar spectrum (Fig. 1c). The hourly course of keratitis-effective irradiance in different seasons was calculated in order to determine the radiant exposure of clinically observed cases of keratitis solaris.

**Fig. 1.** (a) Action spectrum of keratitis. (b) Spectrum of global radiation for different solar elevations at an ozone content of 0.24 cm (unbroken line) and 0.40 cm (broken line). Both curves are identical for λ > 340 nm. (c) Keratitis-effective spectrum. The curves apply to different solar elevations, with unbroken lines for 0.24 cm ozone and dotted lines for 0.40 cm.