Color Vision Measured With Pseudoisochromatic Plates at Five-and-a-Half Years in Eyes of Children From the CRYO–ROP Study

Velma Dobson,* Graham E. Quinn,† Israel Abramov,‡ Robert J. Hardy,§ Betty Tung,§ R. Michael Siatkowski,|| and Dale L. Phelps¶

Purpose. To investigate the prevalence of color deficits at age 5 1/2 years in preterm children with birth weights of less than 1251 g who participated in the multicenter Cryotherapy for Retinopathy of Prematurity (CRYO-ROP) study.

Methods. Two cohorts of CRYO-ROP participants served as subjects: 1055 children who participated in a long-term study of the natural history of ROP at 5 of the 23 CRYO-ROP centers, and 187 children (from all 23 study centers) who had threshold ROP in both eyes and who were randomized to receive cryotherapy in 1 eye. Monocular color vision was tested at age 5 1/2 years, using the Standard Pseudoisochromatic Plates, part 2 (SPP2) for acquired color vision defects.

Results. In the Natural History cohort, prevalence of red–green (R–G) color deficits was 6.6% for males and 1.0% for females, similar to that of the general adult population. Prevalence of blue–yellow (B–Y) color deficits was 2.8% for males and 2.2% for females, more than 200 times that in the general adult population. Prevalence of B–Y deficits was not related to birth weight, gestational age, acute-phase ROP, optic atrophy, or retinal residua of ROP, but was related to visual acuity. In the Threshold ROP cohort, color vision deficits were no more likely in eyes that had received cryotherapy than in control eyes.

Conclusions. The results confirm an increased prevalence of B–Y deficits in children born before term, and provide evidence that the increased prevalence is not related to birth weight, gestational age, or severity of ROP within this group of preterm children. No evidence was found to indicate that cryotherapy increased the rate of color vision deficits in eyes with threshold ROP. Invest Ophthalmol Vis Sci. 1996;37:2467–2474.

Abramov et al12 reported that preterm children cared for in the neonatal intensive care unit (NICU) are at risk for tritanlike (or blue cone) color vision deficits. Abramov et al12 hypothesized that the increased prevalence of tritanlike deficits was because of high-intensity constant ambient illumination that was present in the NICU in which the infants were treated. This hypothesis is supported by evidence from the animal literature indicating that blue cones are especially prone to light-induced damage and that they recover less well than do the other cone types.3,4 Other potential explanations for color vision deficits in preterm infants include photoreceptor damage related to retinopathy of prematurity (ROP) and changes in color vision related to optic atrophy or glaucoma. However, the contribution of these factors could not be addressed in the studies of Abramov et al, due to a small sample size, and lack of detailed perinatal and ocular information for the population.

Another potential cause of color vision deficits in today’s NICU graduates is cryotherapy, a surgical treatment for severe ROP. Cryotherapy involves abla-
tion of the retina peripheral to abnormal vascular proliferation and, although it is beneficial to both retinal structure and visual function in the long term, it may be associated with acute inflammation in the eye during the postoperative period. Because the effect of inflammation and necrosis of the peripheral retina on the remaining developing retina is not known, there is concern about the long-term effect of cryotherapy on photoreceptor function.

As part of the Cryotherapy for Retinopathy of Prematurity (CRYO-ROP) study, visual acuity, color vision, and ocular status were assessed at age 5 1/2 years in more than 1000 preterm infants with birth weights less than 1251 g. More than 200 of these infants were participants in the randomized trial of cryotherapy, in which infants in whom severe (threshold) ROP developed in both eyes during the neonatal period had one eye assigned randomly to receive cryotherapy and one eye assigned to serve as a control eye. Infants in the randomized trial in whom severe ROP developed in one eye had that eye randomized to cryotherapy or to serve as a control eye. The purpose of this report is to present the color vision results of children in the CRYO-ROP study and to examine the relation between color vision deficits and ocular and perinatal variables in this population.

METHODS

Subjects

All subjects were participants in the CRYO-ROP study. The study was conducted in accord with the tenets of the Declaration of Helsinki and was approved by the human experimentation committee of each participating institution. Informed consent was obtained from each subject’s parent or guardian before testing.

Natural History Cohort. The Natural History cohort consisted of 1055 children who had color vision tested at age 5 1/2 years as part of a long-term follow-up study of the natural history of ROP and ocular development of CRYO-ROP study participants. These subjects comprised 87.3% of the 1208 children who were observed of CRYO-ROP study participants. These data were included in analyses only if, before testing, refractive error had been corrected and amblyopia treated according to study protocol. After acuity and color vision assessment, a study ophthalmologist conducted an eye examination.

Threshold Retinopathy of Prematurity Cohort. The Threshold ROP cohort consisted of 187 children in whom threshold ROP developed in both eyes during the perinatal period, and in whom one eye was assigned randomly to receive cryotherapy and one eye assigned to serve as a control eye. The children, all of whom had color vision tested at age 5 1/2 years as part of the long-term follow-up study, comprised 97.9% of the 191 randomized subjects in whom threshold ROP developed in both eyes and who participated in the 5-1/2 year study examination at the 23 study centers.

Color Vision Testing Procedure

The Standard Pseudoisochromatic Plates, Part 2 (SPP2), for acquired color vision deficiency (Igaku Shoin, Tokyo, Japan) were used for testing color vision. During testing, the child was seated at a table, with the color plates illuminated by a Verilux full-spectrum fluorescent lamp (F15T8/VLX). The room lights were turned off. Test distance was 75.0 cm but could be decreased to 37.5 or 19.0 cm if needed because of poor visual acuity or poor attention. The right eye was tested first, followed by testing of the left eye. Optical correction, if prescribed, was worn during testing.

Initially, the child was shown the first two SPP2 plates (demonstration plates) and asked to identify or trace the numbers on each plate. Only children who completed this task were tested further. During the test phase, the child was asked to identify or trace the numbers on each of the 10 test plates.

Visual Acuity Testing and Eye Examination

Monocular recognition acuity was assessed using the Distance Early Treatment for Diabetic Retinopathy Study Snellen chart as described previously. Standard test distance was 4 m; however, children who would not respond at the 4-m distance could be tested at a closer distance (1.0, 0.5, or 0.25 m). Acuity was estimated as the smallest letter size that the child could identify correctly on three of five presentations. Acuity data were included in analyses only if, before testing, refractive error had been corrected and amblyopia treated according to study protocol. After acuity and color vision assessment, a study ophthalmologist conducted an eye examination.
TABLE 1. Order of Presentation of SPP2 Color Plates

<table>
<thead>
<tr>
<th>Plate Number</th>
<th>Diagnostic Category*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Demonstration</td>
</tr>
<tr>
<td>2</td>
<td>Demonstration</td>
</tr>
<tr>
<td>3</td>
<td>B-Y B-Y</td>
</tr>
<tr>
<td>4</td>
<td>B-Y B-Y</td>
</tr>
<tr>
<td>5</td>
<td>B-Y N</td>
</tr>
<tr>
<td>6</td>
<td>B-Y N</td>
</tr>
<tr>
<td>7</td>
<td>B-Y N</td>
</tr>
<tr>
<td>8</td>
<td>B-Y R-G</td>
</tr>
<tr>
<td>9</td>
<td>B-Y R-G</td>
</tr>
<tr>
<td>10</td>
<td>B-Y R-G</td>
</tr>
<tr>
<td>11</td>
<td>B-Y R-G</td>
</tr>
<tr>
<td>12</td>
<td>R-G N</td>
</tr>
</tbody>
</table>

B-Y = diagnostic for B-Y deficits; R-G = diagnostic for R-G deficits; N = diagnostic for neither B-Y nor R-G deficits.
* Two test numbers/plate.

Data Analysis

Subjects. For the Natural History cohort, we analyzed the data of only one eye per subject to avoid difficulties in analysis that arise because of the correlation between results of fellow eyes. For subjects who had no ROP in both eyes, had no ROP in one eye and ROP that did not progress to threshold severity in the other eye, or had ROP in both eyes that did not progress to threshold severity in either eye, one eye was selected at random for entry into the data analysis. For children who had threshold ROP, data from the control eye (i.e., the eye that did not receive cryotherapy) were used. For analysis of results in the Threshold ROP cohort, both eyes of each subject (the treated eye and the fellow control eye) were included in the analysis.

Diagnosis of Color Vision Deficits. Of the 20 numbers on the test plates, 5 are diagnostic for red–green (R–G) deficits, and 11 are diagnostic for blue–yellow (B–Y) deficits. An eye was scored as color deficient if more than one error was made on the numbers diagnostic for R–G deficits or if more than two errors were made on the numbers diagnostic for B–Y deficits. Less strict criteria were used for B–Y deficits because the left-hand number on plate 3 (refer to Table 1) often is missed by individuals with normal color vision (see analysis of color plates presented in the Appendix).

RESULTS

Natural History Cohort

Success Rates. Of the 1055 children in whom assessment of color vision was attempted, 912 (86.4%) provided color vision results, 38 (3.6%) were untestable due to low vision, and 105 (10.0%) were untestable due to neurodevelopmental delay.

Color Vision Results. Figure 1 presents the overall prevalence of R–G and B–Y deficits in male and female subjects in the Natural History cohort. For the 912 subjects who provided color vision results, 6.6% of males and 1.0% of females had R–G color vision deficits and 2.8% of males and 2.2% of females had B–Y deficits. Although the prevalence of R–G deficits is similar to that reported for adults, the prevalence of B–Y deficits is at least 200 times that of the adult population.

Table 2 lists the prevalence of B–Y deficits as a function of birth weight, gestational age, severity of acute-phase ROP, and level of acuity. Chi-square analysis showed no difference across birth weight or gesta-
tional age groups in the prevalence of B–Y deficits. As listed in Table 2, there is a trend toward a higher prevalence of color vision deficits in eyes of patients with more severe ROP, although none of the 23 children with severe ROP had B–Y deficits. Chi-square analysis indicated no significant relation between severity of ROP and prevalence of B–Y deficits, even when data from eyes with severe ROP were excluded from the analysis.

Optic atrophy, glaucoma, cataract, and retinal disturbance are ocular disorders that have been associated with color vision deficits. However, none of the 912 eyes with color vision data in the Natural History cohort had glaucoma or cataract, and of the 2 that had optic atrophy, neither had a B–Y deficit. ROP residua were present in only 7 of the 912 eyes with color vision data in the Natural History cohort. Three of the seven eyes had abnormally straightened temporal retinal vessels, and four had macular ectopia, but only one (an eye with straightened vessels) showed a B–Y deficit.

It has been reported that there is an increased prevalence of color vision deficits in visually impaired children. As listed in Table 2, the prevalence of B–Y deficits increased with decreasing visual acuity. Chi-square analysis showed a significant difference among acuity categories ($\chi^2 = 49.5, P < 0.0001$), suggesting that eyes with below-normal acuity have more B–Y color vision deficits than do eyes with normal acuity.

Threshold Retinopathy of Prematurity Cohort

Success Rates. Of the 187 children in the Threshold ROP cohort in whom assessment of color vision was attempted, 55 (29.4%) provided color vision results from both the treated and control eye. Fifty (26.7%) provided color vision results from only 1 eye, 42 (22.5%) were untestable because of low vision in both eyes, and 40 (21.4%) were untestable because of neurodevelopmental delay.

Color Vision Results. As shown in Figure 2, results of color vision testing provided no evidence that cryotherapy produced an increase in either R–G or B–Y color vision deficits in the 55 patients (26 males and 29 females) in the Threshold ROP cohort in whom color vision results were obtained from both eyes. The difference in the prevalence of R–G color deficits between treated and control eyes was 0.0%, with 95% confidence limits −6.1% to 6.1%. In two patients (3.6%, one male and one female), both the treated and control eyes showed R–G color deficits, whereas in one patient (1.8%, a male), only the treated eye showed an R–G deficit, and in one patient (1.8%, a female), only the control eye showed an R–G deficit. The difference in the prevalence of B–Y color deficits between treated and control eyes was −3.6%, with confidence limits −2.5% to 9.7%. One patient (1.8%, a male) showed a B–Y deficit in both the treated and the control eye, whereas two patients (3.6%, both females) showed a B–Y deficit in only the control eye.

The sample size of 55 was too small to evaluate the difference between treated and control eyes. Nevertheless, the confidence limits suggest that the sample size was adequate to detect large shifts (6.1% for R–G deficits and 9.7% for B–Y deficits) in the prevalence if they were present.

DISCUSSION

The CRYO-ROP study population is composed of a large cohort of children with birth weights less than 1251 g, who were born between January 1, 1986, and November 30, 1987, and who are now reaching school age. There are two reasons to examine color vision in this group of children who were high-risk preterm infants. First, Abramov et al reported an increased prevalence of blue-yellow color deficits among infants treated in the NICU. Second, because cryotherapy is an ablative procedure, it might produce photoreceptor or other structural changes in adjacent retina that could affect color vision. Among the large group of subjects in the Natural History cohort, the prevalence of R–G deficits (6.6% for males and 1.0% for females) was similar to that reported for the general adult population (8% for males and 0.5% for females). In contrast, the prevalence of blue-yellow deficits (2.8% for males and 2.2% for females) was more than 200 times that of the general adult population (approximately 1 in 10,000). These findings are in agreement with results reported previously for a small group of preterm children and are consistent
with results indicating that in adults, short-wavelength-sensitive cones are more sensitive to insult and injury than are the middle- and long-wavelength-sensitive cones.\textsuperscript{3,4} Data from the Threshold ROP cohort, a subset of 55 patients with bilateral threshold ROP who had received cryotherapy in 1 eye, showed no detectable difference in the prevalence of either R–G or B–Y deficits when data from eyes that received cryotherapy were compared with data from fellow eyes that had served as control eyes. Thus, the findings do not support the hypothesis that cryotherapy produces photoreceptor changes that lead to abnormal color vision, although it is possible that cryotherapy produced subtle changes in the prevalence of abnormal color vision that could not be detected with a sample size of 55.

The finding that the prevalence of B–Y deficits is greater in the NICU population than in the general population suggests that these are acquired, rather than congenital, deficits. Abramov et al\textsuperscript{1,2} hypothesized that the increased prevalence of B–Y deficits in the NICU population was related to phototoxicity at the photoreceptor level, because they found no B–Y deficits among children who received phototherapy and who, as a result, had their eyes patched for a portion of their stay in the NICU.

The phototoxicity hypothesis could take two forms:

1. A constant high level of illumination may have a cumulative toxic effect on the preterm infant’s developing photoreceptors.
2. A constant high level of illumination during a shorter, critical period of photoreceptor development may disrupt photoreceptor maturation.\textsuperscript{3,4}

The first form of the hypothesis would predict that infants who remain in the NICU longer would show a higher proportion of acquired B–Y color deficits than infants who had shorter stays. We did not have data on the duration of each child’s stay in the NICU; however, two factors that would be expected to correlate with duration of hospital stay are birth weight and gestational age. Our results indicated that there was no relation between prevalence of B–Y deficits and either birth weight or gestational age within this cohort. Thus, there is no clear relation between indicators of duration of NICU stay and prevalence of color vision deficits in this group of children, all of whom spent several weeks in the hospital.

Evaluation of the second form of the hypothesis, that phototoxicity results from a photic insult during a specific time during development, would require knowledge of specific lighting practices in individual nurseries. The CRYO-ROP study was not designed to study this issue, and therefore data on specific lighting conditions were not recorded.

Because photoreceptors are immature in infants,\textsuperscript{21,22} we hypothesized that ROP, and cryoablation that is used in the treatment of severe ROP, could disrupt photoreceptor development and result in color vision deficits. However, our results indicated no relation between prevalence of color deficits and presence or severity of ROP and no measurable increase in color deficits in eyes that had undergone cryotherapy.

In the adult population, acquired B–Y deficits often are associated with ocular disorders, such as glaucoma, optic atrophy, cataract, or macular degeneration.\textsuperscript{14–19} Although optic atrophy, glaucoma, and cataract were more prevalent in the CRYO-ROP population than in the general population, these conditions were nevertheless infrequent.\textsuperscript{5,25,24} In the current report, no children with glaucoma or cataract and only two children with optic atrophy were able to complete color vision testing. Therefore, it is unlikely that the increased prevalence of B–Y deficits observed in participants in the CRYO-ROP study is because of nonretinal causes.

Kalloniatis and Johnston\textsuperscript{20} reported that many children with low vision have color vision deficits. In the present study, the prevalence of B–Y deficits among children with Snellen visual acuity poorer than 20/40 was significantly greater (10.5%, 10/97) than it was among children with visual acuity of 20/40 or better (1.4%, 11/785), in agreement with Kalloniatis and Johnston’s findings. It is unlikely that reduced acuity were the cause of color vision deficits, it would be expected that both R–G and B–Y color vision results, not B–Y results alone, would have been affected. Therefore, it appears that the relation between reduced acuity and B–Y deficits is associative rather than causal. Finally, reduced visual acuity was not the only factor associated with B–Y deficits, because the 1.4% prevalence of deficits among children with normal visual acuity is still more than 100 times that of the general adult population.

The outcome of color vision testing also can be influenced by characteristics of the test used and the conditions under which testing is conducted. Calibration of the chromaticity coordinates of the stimulus spots on each of the SPP2 test plates under the illuminant used during testing (Verilux bulb) indicated that the R–G and the B–Y diagnostic stimuli fell along the deutan–protan and tritan CIE confusion lines, respectively (see Appendix). Therefore, the SPP2 plates, under the illuminant used during testing of
the CRYO-ROP population, are diagnostic for R–G and B–Y color deficits.

Another test characteristic that could influence color vision results is the difficulty of the task required. It has been reported that young children make more errors than do adults on both R–G and B–Y stimuli in color vision tests due to the difficulty of the task. In our results, the prevalence of R–G deficits was in the expected range for adults, whereas the prevalence of B–Y deficits far exceeded that in the general adult population. Thus, one would have to argue that the prevalence of B–Y deficits far exceeded the prevalence of R–G deficits, indicating that the 2% prevalence of R–G deficits found in the CRYO-ROP population simply might reflect the prevalence that would be found if a large number of full-term 5- to 6-year-old children were tested. We cannot rule out this possibility, although we think it extremely unlikely. This might be due to the different categories of genetic dichromats and different rules that are used to define dichromats, assuming correct testing protocol. (See Appendix for calibration details.) As already noted, the relative discriminability of the stimuli on plates designed to identify R–G deficits generally is the same as the relative discriminability of those designed to identify B–Y deficits. A final piece of evidence indicating that the B–Y deficits observed in preterm children are true color vision deficits comes from the studies conducted by Abramov et al.1,2,13 In these studies, tritanlike (B–Y) deficits identified in preterm children tested with standard adult color vision tests were confirmed by showing that these subjects also had significantly elevated thresholds for detecting a stimulus designed to measure the sensitivity of blue cones when the other cone types were suppressed. No cases were found of children who passed the standard color screening but still had elevated blue cone thresholds.

In conclusion, the results of color vision testing of CRYO-ROP participants at age 5 ½ years showed a prevalence of R–G color deficits similar to the prevalence reported in the general adult population. However, prevalence of B–Y deficits was approximately 2%, which is far greater than the prevalence of approximately one in 10,000 that has been reported for the general adult population. This result confirms, in a large sample of children, Abramov et al.'s3,12,13 observation that preterm infants treated in the NICU are at risk for color vision deficits involving the B–Y pathways. Post-hoc analyses indicated that, in this sample of preterm infants with birth weights less than 1251 g, the B–Y deficits were not related to birth weight, gestational age, severity of ROP, or presence of ocular abnormalities at age 5 ½ years. The data suggest, however, that B–Y deficits are more prevalent in children with reduced acuity.

The results of the Threshold ROP cohort provided no evidence that cryotherapy increases the prevalence of color vision deficits in children who had severe ROP in the neonatal period. This suggests that either photoreceptors in the surviving retina that are
disturbed during cryotherapy recover sufficiently to allow normal color vision, or that enough developing photoreceptors are left undisturbed by cryotherapy to allow normal color vision in the eye. More specialized color vision testing would be required to determine whether localized areas of deficient color vision are present in eyes that received cryotherapy in the neonatal period.

**Key Words**
children, color blindness, color vision, cryotherapy, retinopathy of prematurity

**References**

**APPENDIX**
Each of the SPP2 pseudo-isochromatic plates used in this study consists of six different dot types. Ideally,
two of them have the same neutral chromaticity, but differ in luminance (bright versus dark); the background of each plate is a random assortment of these dots. The remaining four, grouped in pairs, also should have the same luminances as the background; however, one pair should have a chromaticity that differs from the background, whereas the other pair has still another chromaticity. These dots are used to create the numerals the subject is asked to identify. For diagnostic purposes, the difference between the chromaticity of any numeral and the background should lie, on a chromaticity diagram, along one of the confusion lines that typifies the behavior of subjects who have a given class of color vision defect.

A potential problem in any color vision measurement is the particular type of fluorescent lamp used to illuminate the plates; these lamps have prominent lines in their emission spectra that could, in principle, bias the spectra of the test elements. Therefore, the plates were set up and illuminated as they would have been during vision testing (refer to text). A scanning spectro-radiometer (Spectrascan model PR-703A/PC; Photo Research) was placed where a subject's eye would have been; this instrument was used to measure the spectra of representative dots from the background and numerals of each of the plates; the results were converted to photometric units of luminance and chromaticity.

Across all the plates, the two luminances (bright versus dark dots) were similar; within a subset of any pair (i.e., the bright or the dark dots), they differed unsystematically by approximately 0.1 log unit; thus, luminance variations should not have been sufficient to support detection of any of the numerals. The chromaticity coordinates of the background dots were similar across plates and clustered tightly about an achromatic locus close to that of an equal-energy stimulus. The chromaticities of the pairs of dots associated with any one of the test numerals also were similar; furthermore, each numeral's chromaticity differed by a small but clear amount from that of the background. Thus, each numeral should have been detectable by an attentive subject with normal color vision; the one exception, mentioned in the text, was the left-hand numeral on plate 3 whose chromaticities were minimally different from the background. All the chromaticities of numerals that were reported to be diagnostic of an R–G deficit fell into two tight clusters on either side of a line passing through the chromaticity locus of the neutral background. The slope of the line more closely matches that of one of the confusion lines of a deuteranope; however, the separations of the chromaticities are sufficiently small that they also should be acceptably close to a protanope's confusion line; thus, the plates should have been appropriate to identify those with congenital R–G deficits. More important for this article, the chromaticities of the numerals claimed to detect B–Y deficits also clustered on either side of a line passing through the locus of the background chromaticity. This line agrees well with a tritanope's confusion line, specifically, a line from 400 to approximately 568 nm on the spectrum locus of the chromaticity diagram. These plates, with the one exception noted, should have been appropriate to identify the B–Y deficits associated with reduction in responses of short-wavelength-sensitive cones.