Carbon Disulfide Effects on the Visual System

I. Visual Thresholds and Ophthalmoscopy

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The visual effects of carbon disulfide exposure were studied in macaque monkeys with measurements of visual thresholds, fluorescein angiography and fundus photography. Five monkeys were exposed by inhalation for 6 hr a day, 5 days a week to 256 ppm carbon disulfide (CS₂). The motor dysfunction observed in these monkeys appeared to be entirely reversible. All five suffered severe reductions in visual acuity and contrast sensitivity although flicker resolution was not affected. Visual loss was found to be irreversible, with degeneration of substantial numbers of retinal ganglion cells (companion paper) in those monkeys permitted to survive after the termination of exposure. None of the monkeys developed retinal microaneurysms or hemorrhages, major accepted signs of visual toxicity in CS₂ exposed humans; thus, permanent visual loss may result from carbon disulfide exposure even in the absence of retinal vascular effects. Invest Ophthalmol Vis Sci 29:512–518, 1988

Carbon disulfide is a widely used solvent in industry and agriculture, with the most severe human exposures occurring in the manufacture of viscose rayon.¹ In the U.S. alone approximately 20,000 workers are occupationally exposed to this chemical.² The toxicity of carbon disulfide has been recognized for many years² and is currently diagnosed in part by retinal vascular changes or losses of visual sensitivity. The presence of retinal microaneurysms, hemorrhages or exudates are common criteria for the diagnosis of human carbon disulfide toxicity. Such signs have not been observed in some groups of exposed workers, for example those from Finland; however, workers without frank vasculopathy may show other signs of disturbed retinal vascular function, such as peripapillary underfilling during fluorescein angiography.³ Carbon disulfide also causes coronary and renal vascular disease, but retinal effects have proven more useful for differential diagnosis because they are easily visualized.

Loss of visual function has been an important adjunct in the detection of carbon disulfide toxicity. Early reports of human carbon disulfide toxicity described central scotoma (a region of severe visual loss) and depressed sensitivity in the peripheral visual field accompanied by optic atrophy.⁴ Other visual deficits found in carbon disulfide exposed workers include pupillary disturbances,⁵ blurred vision,⁶ and impaired color vision.⁷ Although awareness of the danger of carbon disulfide has resulted in less severe human exposures, visual loss remains one of its major effects.

The present study examined visual function and the condition of the retinal vasculature using behavioral techniques and fluorescein angiography respectively, and related these to histological changes.⁸ Macaque monkeys were exposed to carbon disulfide by inhalation, 6 hr a day, 5 days a week at 256 ppm, a concentration approximately 13 times the current federal standard time-weighted average for 8 hr exposure (20 ppm) and two and a half times the recommended peak exposure (100 ppm).² All exposed monkeys showed reversible motor impairment² and severe and irreversible impairment of visual sensitivity. However, none of the monkeys showed evidence of the development of retinal microaneurysms or hemorrhages. The morphological correlates of these findings, especially degeneration of retinal neurons, are described in the companion paper.⁸

Materials and Methods

Subjects

Six feral, young adult female macaque monkeys (Macaca nemestrina) were studied. Each monkey

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Preliminary reports of this research were presented at the 1983 (March 7–11, Las Vegas, Nevada) and 1985 (March 18–22, San Diego, California) meetings of the Society of Toxicology and the 1985 meeting of the Association for Research in Vision and Ophthalmology, May 6–10, Sarasota, Florida.

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had free access to Purina monkey chow and received fresh fruit regularly. The monkeys were water-deprived for approximately 14 hr before daily behavioral testing, 5 days each week. After testing they were exposed to carbon disulfide by inhalation (see below) for 6 hr, and subsequently given free access to water for about 3 hr. The control monkey (702) had the same daily regimen except that sham exposure was substituted for the 6 hr carbon disulfide exposure. Eyes of all monkeys were refracted by retinoscopy before exposure, and none showed a spherical error or astigmatism of greater than 0.5 diopter. Use of the monkeys conformed to the ARVO Resolution on the Use of Animals in Research.

Visual Thresholds

Apparatus and procedures have been described previously. Briefly, the monkeys were restrained in an acrylic chair, binocularly viewing two high resolution display oscilloscopes (Tektronix 606 with P31 phosphor; Beaverton, OR) at a distance of 114 cm. The oscilloscope screens measured 8 X 10 cm (4 x 5 deg visual angle) and each was bordered by a 24 cm surround matched to the displays in color and luminance. The centers of the two screens were 30 cm apart. The mean luminance of the visual stimuli was always 17 cd/m². A single test stimulus was presented on one of the two displays on each trial; the monkey responded to the location of this stimulus (under a spatial forced-choice procedure) by pressing the corresponding one of two buttons located on a response panel. Each session consisted of 200 trials.

Three types of visual thresholds were measured before, during and after the period of carbon disulfide exposure: contrast sensitivity as a function of spatial frequency, visual acuity and flicker fusion frequency. Contrast sensitivity was measured with stationary vertical gratings with sinusoidal horizontal luminance profiles, ranging in spatial frequency (reciprocal of the width of a light and dark bar) from 0.4 to 23 cycles/deg of visual angle. In each session a single spatial frequency was used. Grating contrast was defined as (Lₘₐₓ - Lₘᵢₙ)/(Lₘₐₓ + Lₘᵢₙ), where Lₘₐₓ and Lₘᵢₙ are the highest and lowest luminances in the grating. Onset of contrast sensitivity remained constant (0.55) and spatial frequency was varied in 0.18 octave steps. Flicker fusion frequency was measured in a similar manner except that the stimulus was unpatterned flicker of sinusoidal temporal wave form. Modulation depth of the flicker (Lₘₐₓ - Lₘᵢₙ)/(Lₘₐₓ + Lₘᵢₙ) was 0.55 and flicker frequency was adjusted (in 0.18 octave steps) according to a staircase to obtain thresholds for flicker detection. Onset of flicker modulation or grating contrast was modulated by one-half cycle of a raised cosine of period 1.6 sec (0.625 Hz). When testing acuity and flicker sensitivity, luminance cues were avoided by using an intertrial stimulus of the same contrast, but of supraresolution spatial and temporal frequency, respectively.

Exposure to Carbon Disulfide

The experimental monkeys were exposed to an atmosphere containing 256 ppm carbon disulfide in a hexagonal exposure chamber, 6 hr per day, 5 days per week. Two monkeys at a time were placed in the exposure chamber in a 39 X 39 X 49 cm holding cage early in the morning, just after the completion of visual testing. A control monkey was placed in the chamber in the early afternoon after visual testing, for a 6 hr sham exposure. Some animals had two series of exposures; the duration of each series and the interval between them are presented in Table 1.

Carbon disulfide vapor was generated by sparging the solvent (Eastman, Rochester, NY) with a small stream of nitrogen. This saturated vapor stream was added to the intake of the exposure chamber, which was maintained at a negative pressure. The carbon disulfide concentration was monitored continuously.
with an infrared spectrophotometer (Foxboro-Wilks MIRAN; South Norwalk, CT); the spectrophotometer was connected to a servo-controlled nitrogen metering valve. This servomechanism compensated for small variations in chamber airflow and solvent temperature and level, and served additionally as a safety device. Concentrations were maintained within 5% of the nominal concentration.

**Results**

The visual acuity for all monkeys was examined across the duration of the repeated exposure protocol, and is shown in Figure 1. Sham exposure (702) produced little effect on acuity. Visual acuity was profusely reduced in monkeys 703, 910 and 115, but showed little change in monkey 114 during the first 36 days of exposure to carbon disulfide. The reduced acuity resolved rapidly and completely in monkey 115, slowly and partially in 910, and persisted in monkey 703. A second, longer period of carbon disulfide exposure for monkeys 114 and 115 resulted in greatly reduced visual acuity in both monkeys, with partial recovery in 114 and slight recovery in 115. Finally, monkey 113 had a slow onset of impaired acuity (beginning after about 10 weeks of exposure), but this monkey's acuity was greatly reduced by the time of sacrifice at the end of the exposure series.

The flicker resolution of the exposed monkeys (not shown) was little affected by carbon disulfide, showing slight and transient decreases only in monkeys 910, 114 and 115 near the end of the first exposure period. However, psychometric functions remained after the termination of exposure. Additionally, final photographic measures were made 3 months after the end of the exposure for monkeys 703 and 910 and 5 months after exposure in monkeys 114 and 115. Each monkey was sedated for photography with intramuscular injections (15 mg/kg as needed) of ketamine hydrochloride (Ketaject®; Bristol Labs, Syracuse, NY). Mydriasis and cycloplegia were achieved with two drops of phenylephrine and cyclopentolate (Cy- clymydril®; Alcon, Fort Worth, TX) instilled twice in each eye. Color fundus photographs were made under white- and red-free illumination with a Zeiss Fundus Flash II camera (Kodachrome ASA25 film; Kodak, Rochester, NY). Serial fluorescein angiograms (transit time of 12 to 15 min) were then made of each eye separately after the rapid intravenous infusion of 0.5 ml of 10% sodium fluorescein. At least 20 min were allowed to elapse between completion of transit in one eye and injection of fluorescein for photography of the fellow eye, to minimize background fluorescence from the previous injection.

**Blood Levels of Carbon Disulfide**

Five milliliter samples of venous blood were obtained at least once during exposure or sham exposure from each of the experimental and control animals. Samples were taken within 3 min of the end of a daily exposure session and analyzed for free and acid-labile carbon disulfide by the method of Lam and DiStefano.11

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**Fluorescein Angiography and Fundus Photography**

Fluorescein angiography and color fundus photography were performed on each monkey before the exposure series commenced, during exposure and
steep and shifted toward lower frequencies, indicating that this short-lived effect represented visual loss and not behavioral disruption.

Contrast sensitivity of the monkeys was severely degraded by carbon disulfide exposure, but the time course of this effect did not completely parallel that of visual acuity. Contrast sensitivity was always reduced whenever visual acuity was impaired (see below for examples). However, there were periods during the testing of monkeys 114 and 115 when visual acuity was almost fully recovered, but contrast sensitivity remained substantially lowered. This result was especially prominent during the two intervals whose midpoints are marked in Figure 1 by the letters a and b. The contrast sensitivity during these intervals is compared in Figure 2 to the sensitivity of the monkeys before dosing, and both monkeys show a substantial loss of contrast sensitivity. In contrast, the visual acuity of each monkey (horizontal location of rightmost points on the two graphs) was close to normal at this time.

However, it can be seen in Figure 1 that the acuity of both of these monkeys later decreased, that of 114 without further dosing, and that of 115 during a later dosing period. Thus, at the time of sacrifice, neither of these monkeys showed the selective sparing of visual acuity that is evident in Figure 2.

The final contrast sensitivity of all monkeys is shown in Figure 3. The control monkey (702) had no change in sensitivity as a result of sham exposure. The other four monkeys had substantial losses across

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**Fig. 2.** Contrast sensitivity as a function of spatial frequency of the test grating for two monkeys before CS₂ (circles) and at the time marked by a and b in Figure 1 (squares). These curves illustrate an instance of contrast sensitivity loss at a time when visual acuity (rightmost data point in each plot) had recovered to almost normal values. The approximate time of the latter measures is marked in Figure 1 by the letters a and b. Each data point represents the mean of three sessions ± SD.

**Fig. 3.** Final contrast sensitivity as a function of spatial frequency for four carbon disulfide exposed and one control (702) monkey. Circles show contrast sensitivity before carbon disulfide exposure, triangles, that after the first but before the second exposure, and squares, the level at which sensitivity stabilized after a single period of exposure in monkeys 703 and 910, and after two periods of exposure for monkeys 114 and 115.
Fig. 4. Venous phase fluorescein angiogram of the right eye of monkey 114 after two periods of carbon disulfide exposure. The optic disc is seen at the left as the area of intense fluorescence and the fovea at the right as the vessel-free area. No microaneurysms were seen in the series of angiograms of each eye of all CS₂ exposed monkeys.

all spatial frequencies, but especially at middle and higher spatial frequencies.

Fluorescein Angiography and Fundus Photography

Multiple fluorescein angiograms taken 3-5 months after the end of CS₂ exposure were compared to those from the same eyes before and during dosing for the development of hemorrhages, microaneurysms, constriction of arterioles or other abnormalities; none were seen. Figure 4 shows an example of an angiogram of one monkey (No. 115) after two periods of carbon disulfide exposure, illustrating normal central retinal vasculature. Marked impairment of visual function was apparent at the time. We could not detect delayed peripapillary filling. Neither reduction of fine vessels nor decreased fluorescence in the optic nerve could be demonstrated with blind ratings.

Color fundus photographs also showed no evidence of hemorrhages, exudates, microaneurysms or constriction of the retinal arterioles. We did not have sufficient control photographs to determine if attenuation of small vessels in the optic nerve head, or pallor in the temporal portion of the optic disc, accompanied the loss of visual function.

Blood Levels of Carbon Disulfide

Blood levels of acid-labile carbon disulfide were measured at least once for each monkey during exposure. Samples from monkeys 910 and 703 were taken after 35 days of exposure and contained 14.35 and 10.41 µg/ml, respectively. Levels for monkeys 113 after 49 and 84 days exposure were 21.76 and 22.1; monkey 114 after 34 days of the first exposure, 20.02, and after 45 and 80 days of the second exposure, 18.44, and 22.4; monkey 115 after 34 days of the first exposure, 15.33 µg/ml. Free carbon disulfide was not measured in blood under these exposure and analytical conditions.

Discussion

Carbon disulfide exposure resulted in a devastating loss of visual acuity in all five exposed monkeys, although with much variation in time course. Despite a partial recovery from the initial loss, all four monkeys suffered permanent visual impairment for the several months they survived after the termination of exposure. Contrast sensitivity was profoundly reduced even during the period when partial recovery of acuity was observed in two monkeys. Flicker resolution, which is dependent on intact functioning of the periphery of the retina, was only transiently disrupted. Results for one monkey (115) suggest that even severe acuity loss may be reversible if exposure is terminated in time. The severe and permanent visual losses observed in all monkeys were consistent with postmortem findings of the degeneration of substantial numbers of retinal ganglion cells, especially in the central retina. Retinal vascular disorders, which are often associated with carbon disulfide exposure in humans, were not found in any of the exposed monkeys, indicating that carbon disulfide can cause neuronal damage in the retina without ophthalmoscopic, angiographic or histological evidence of vascular involvement.

Relation of Functional to Morphologic Changes

Two features of the morphologic findings can be related to the visual dysfunction observed. The profound loss of central ganglion cells in three of the four monkeys resulted in a sharply circumscribed region of fiber loss in the optic nerve. Such a severe loss of ganglion cells in the central retina should result in little ability to see (dense scotoma) in that part of the visual field with a resulting eccentric locus of fixation.
In this respect, the carbon disulfide-induced lesion is similar to those produced by argon laser photoocoagulation of the central retina.\textsuperscript{10} Such a lesion differs from that in the present study by involving photoreceptors more than ganglion cells, but provides a clear model of visual loss in a small region of the retina.

Visual loss after such a lesion is confined almost exclusively to higher spatial frequencies with visual acuity dropping as it does after carbon disulfide. On the other hand, the laser lesion produces little effect on lower spatial frequencies; in this respect, the pattern of visual loss is very different from that seen with CS\textsubscript{2} exposure. The severe acuity loss of monkey 115 indicates that there was severe damage to central ganglion cells, although the morphological evidence presented in the companion paper\textsuperscript{8} shows that this damage was less severe than in the other three monkeys.

Our morphological findings, including cytochrome oxidase histochemistry and horseradish peroxidase transport,\textsuperscript{4} indicated a more substantial effect on retinal ganglion cells projecting to parvocellular layers of the lateral geniculate nucleus,\textsuperscript{14} than on those projecting to magnocellular layers. Carbon disulfide exposure produces effects similar to acrylamide in this respect,\textsuperscript{15} although acrylamide causes a more selective degeneration of retinal ganglion cells projecting to parvocellular layers. Other evidence\textsuperscript{16} also indicates that carbon disulfide and acrylamide produce different patterns of toxic effects. Monkeys in the present study did show great losses in contrast sensitivity at low spatial frequencies, like those produced by acrylamide.\textsuperscript{17} The fact that acrylamide causes low spatial frequency loss suggests that this portion of the visual impairment of monkeys in the present study may be accounted for entirely by damage to parvocellular projecting ganglion cells.

Flicker-fusion frequency was transiently affected. Under the stimulus conditions of the present study, this visual function is mediated by the periphery of the visual field in macaques\textsuperscript{10} and humans.\textsuperscript{18,19} Sparing of this function was thus consistent with the observed sparing of peripheral retinal ganglion cells. Preservation of flicker sensitivity provides additional evidence that the losses in spatial vision were not due to nonspecific behavioral, pupillary or eye movement disturbances.

An early effect of carbon disulfide exposure in two monkeys, illustrated in Figure 2, was a depression of contrast sensitivity with sparing of visual acuity (rightmost data point). This is an unusual effect (visual acuity is typically reduced in optic nerve disease), but a similar result has been observed in some cases of multiple sclerosis.\textsuperscript{20} The sparing of visual acuity suggests that at the time of these measurements ganglion cells in central retina were not yet severely damaged. It is possible that this effect indicates selectivity not to middle and low spatial frequencies, but to the high contrast sensitivity that is found in those frequencies. Such selectivity would be consistent with reduced numbers of active ganglion cells with a corresponding decreased probability summation between cells,\textsuperscript{21} producing the result in Figure 2.

Relation to Visual Effects of Carbon Disulfide in Humans

Our findings are consistent with early reports of central scotoma (loss of vision in the central part of the visual field) and paleness or atrophy of the optic disk.\textsuperscript{5} It is likely, on the basis of our morphological findings in the companion paper,\textsuperscript{8} that monkeys 703, 910 and 114, and possibly 115, would have shown central scotomata, if visual fields had been measured in this study.

Of the visual defects most often reported in carbon disulfide exposed workers,\textsuperscript{22,23} impaired macular sensitivity and dark adaptation are most likely related to the ganglion cell damage seen in this study. Lewin and Guillery\textsuperscript{24} reported that long-term carbon disulfide exposure greatly impaired color vision, especially in the central part of the visual field. More recently Raitta et al\textsuperscript{7} have measured impairment of color vision in carbon disulfide exposed workers using the Farnsworth-Munsell 100-Hue Test. The color vision disturbances reported in both of these studies could well be due to adverse effects on retinal ganglion cells, especially those projecting to parvocellular layers of the lateral geniculate nucleus. These cells have prominent color-opponent physiological responses, and were most susceptible to carbon disulfide exposure in the present study. It is likely that visual system damage in the workers studied by Raitta et al\textsuperscript{7} was much less advanced than that in the monkeys of the present study, as they reported no acuity loss. Their results suggest that color vision may be a good indicator of mild carbon disulfide toxicity. Our findings suggest that contrast sensitivity should be examined in all workers exposed to carbon disulfide.

Use of Visual Testing to Detect Carbon Disulfide Toxicity

Visual acuity measures proved useful for detecting the onset of damage and following the time course of carbon disulfide toxicity in this study. Acuity measures showed that the early stages of carbon disulfide-induced visual loss are reversible (monkey 115 first exposure period) while permanent deficits in acuity probably reflected substantial degeneration of gan-
glion cells. Flicker-fusion thresholds were not affected by carbon disulfide, nor is it likely that other measures of peripheral visual capacities would be.

Since contrast sensitivity was sometimes greatly reduced when acuity was unaffected, and was always reduced when acuity was impaired, it appears to be an appropriate indicator of carbon disulfide toxicity that may be useful in detecting subtle toxicity. The broad range of contrast sensitivity measures used in this study were too extensive to be used in the clinic; a less comprehensive index of sensitivity, perhaps measuring only a mid-spatial frequency, might be a sensitive and useful clinical test. The current reliance on examination of the retinal vasculature by ophthalmoscopy or angiography has been demonstrated to be ineffective for the diagnosis of either incipient or severe visual impairment produced by carbon disulfide exposure.

**Key words:** contrast sensitivity, visual toxicity, acuity, carbon disulfide, angiography, macaque monkey

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### References