Carbon Disulfide Effects on the Visual System

II. Retinogeniculate Degeneration

Thomas A. Eskin,* William H. Merigan,t‡ and Ronald W. Wood§

We examined the morphological effects of carbon disulfide exposure on neurons and vasculature of the visual system of macaque monkeys. Five monkeys were exposed to 256 ppm carbon disulfide (CS₂) by inhalation for 6 hr a day, 5 days a week. One monkey, sacrificed immediately after exposure, had numerous axonal swellings in the distal optic tract. Four other monkeys survived the exposure period for at least 1 year and were found to have suffered marked degeneration of central retinal ganglion cells, with little or no effect on other neurons in the retina. No evidence was found for arteriosclerotic or aneurysmal changes, suggesting that visual system injury in primates induced by carbon disulfide exposure is not dependent on the occurrence of structural changes in retinal blood vessels. Invest Ophthalmol Vis Sci 29:519-527, 1988

Carbon disulfide (CS₂) is an important industrial solvent that is known for its toxicity to both vasculature and neurons in the visual system.1 Toxic effects of this chemical remain a serious public health concern even though occupational exposure levels have been greatly reduced in recent years. Among the most intensely studied consequences of carbon disulfide exposure are distal axonopathy in the peripheral and central nervous system and arteriosclerosis in the retina, kidney and cardiovascular system.1 Our interest in the ocular toxicity of carbon disulfide was enhanced by the recent finding that another axonopathic chemical, acrylamide monomer, is especially toxic to retinal ganglion cells.2,3

There are virtually no morphological data on the visual toxicity of carbon disulfide, although clinical observations in exposed humans suggest severe neuronal degeneration.4 While retinal ganglion cell loss has been reported in CS₂-exposed mice2 and rabbits,6 the visual system was not described in the one previous detailed study of CS₂-exposed primates.7 The present study reports histological examination of neurons and vasculature of the visual system in CS₂-exposed monkeys. Filamentous axonal swellings were seen in distal optic tract shortly after an extended dosing period. With longer survival, loss of retinal ganglion cells, especially in central retina, was a consistent finding, with secondary changes at higher levels in the visual pathways. Vascular abnormalities were not observed in the retina or elsewhere in the nervous system. The morphological results of this study can be compared to functional alterations in vision in the same monkeys described in a companion paper.8

Materials and Methods

Subjects

Eight feral, young adult, female macaque monkeys (Macaca nemestrina) were studied. Each monkey had free access to Purina monkey chow and received fresh fruit regularly. Additional details are presented in the companion paper.8 Care of animals conformed to the ARVO Resolution on the Use of Animals in Research.

Exposure to Carbon Disulfide

Five monkeys were exposed to 256 ppm carbon disulfide in a hexagonal exposure chamber, 6 hr per day 5 days per week. Generation of the carbon disulfide atmosphere is described in the companion paper.8 Two monkeys at a time were placed in the exposure chamber, just after the completion of visual testing. Some animals had two series of exposures; the duration of each series and the interval between them are presented in Table 1. The three morphology control monkeys were never exposed to carbon disulfide.
Table 1. CS$_2$ exposures and histopathologic procedures for experimental monkeys

<table>
<thead>
<tr>
<th>Monkey</th>
<th>First exposure (days)</th>
<th>Interval weeks</th>
<th>Second exposure days</th>
<th>Interval to sacrifice (months)</th>
<th>Fixative</th>
<th>TMB for HRP</th>
<th>CO</th>
<th>Paraffin sections</th>
<th>Retinal sections</th>
<th>Ganglion cell counts</th>
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<td>36</td>
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<td>Y</td>
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N = no, Y = yes.

Other Measures

Visual threshold testing, fundus photography, fluorescein angiography and blood carbon disulfide measures were made in experimental monkeys and are described elsewhere.

Horseradish Peroxidase Transport

Three CS$_2$-exposed (Nos. 910, 114, 115) and one control monkey (No. 19) were sedated with 15 mg/kg ketamine and tetracaine ophthalmic ointment (0.5%) was applied to the eye as a local anesthetic. Then 30 µl of 10% wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP) (Sigma Chemical Co., St. Louis, MO) was injected intravitreally in the right eye. All intravitreal injections were made approximately 24 hr before the animals were killed.

Tissue Processing

All experimental and control animals were killed under deep ketamine and barbiturate anesthesia by transcardiac perfusion: 1–2 min of 0.1 M Na phosphate buffer, pH 7.4 followed by 20–30 min of a solution containing 2% formaldehyde (depolymerized paraformaldehyde) and 2.5% glutaraldehyde in 0.1 M Na phosphate buffer (test animals Nos. 703 and 113 and control animals Nos. 109 and 905); or 1–2 min of 0.1 M Na phosphate buffer pH 7.4 followed by 20–30 min of a solution containing 1% formaldehyde and 1.25% glutaraldehyde in 0.1 M Na phosphate buffer and 20–30 min of 5% glycerol in 0.1 M Na phosphate buffer pH 7.4 (test animals Nos. 910, 114 and 115 and control animal No. 19). Brains and eyes were then removed for study. In monkeys Nos. 19, 910, 114 and 115, brains were hemisected: left half-brains were carried through ascending concentrations of glycerol (up to 20%) in 0.1 M phosphate buffer and then frozen and serially sectioned at 40 µm thickness for histochemistry (fixed-frozen sections). The whole brain and both eyes of animals Nos. 703 and 113 and the right half-brain and right eyes in animals Nos. 19, 910, 114 and 115 were immediately immersed in 4% paraformaldehyde combined with 5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4 for 2–4 weeks. Radial sections of right retina, transverse sections of right optic nerve and tract, and right superior colliculus and pretectum, and frontal sections of right lateral geniculate nucleus (LGN) and primary visual cortex were osmicated, embedded in poly 812 epoxy resin (Polysciences, Inc., Warrington, PA), sectioned at 1 µm thickness and stained with paraphenylene diamine for fine histology and morphometric studies. Representative frontal slices from immersion post-fixed right half-brains were embedded in paraffin, sectioned at 4 µm thickness and prepared with hematoxylin and eosin, luxol fast blue periodic acid Schiff for myelin, Bodian for axons, and Cresyl Violet (Nissl) stains.

Retinal Ganglion Cell Counts

Radial sections extending from the fovea along the superior vertical meridian were cut from resin-embedded blocks of retina from animals Nos. 910, 114, and 115 and control animals Nos. 19, 109 and 905. Neurons containing nuclei with visible nucleoli (paraphenylene diamine stain) were counted in two serial sections separated by at least 100 µm. Ganglion cells were counted in successive 375 µm segments along radial sections of retina beginning at the fovea and proceeding vertically to the ora serrata.

Histology

_Tetramethyl benzidine HRP:_ Selected fixed frozen sections from brains of monkeys receiving intracocular WGA-HRP injections were reacted with tetramethyl benzidine (TMB) according to a standard method; briefly: (1) sections were rinsed six times (10 sec each) in distilled water; (2) sections were incubated for 20 min with TMB solution [Solution A:
Fig. 1. Axonal swellings in distal optic tract (arrowheads) of monkey 113.1 μm thick resin, Paraphenylenediamine. Scale bar = 25 μm.

100 mg Na nitroferricyanide (Sigma), 5 ml acetate buffer (pH 3.3) and 100 ml distilled water was mixed with Solution B: 5 mg TMB (Sigma) in 2.5 ml ethanol; (3) 1 to 5 ml 0.3% hydrogen peroxide (volume determined from trial runs on sampled sections) was added rapidly to the mixture of solutions A and B; (4) all sections were incubated another 20 min; and (5) sections were rinsed six times over a 30 min period in acetate buffer (pH 3.3). (All incubations were done under agitation.) Sections were then mounted, air-dried overnight, and coverslipped with Permount® (Fisher Scientific Co., Fairlawn, NJ).

Cytochrome oxidase histochemistry: Selected fixed-frozen sections were prepared for cytochrome oxidase (CO) according to the method of Wong-Riley et al.10 Briefly: sections were placed in an incubation solution consisting of 250 mg diaminobenzidine (Aldrich Chemical Co., Milwaukee, WI), 450 ml 0.1 M phosphate buffer, pH 7.4 and 75–150 mg of cytochrome C (Type III, Sigma). The sections were incubated at room temperature on a shaker with constant monitoring for reaction. The reaction was terminated (usually after about 3 hr) by three rinses in 0.1 M Na phosphate buffer pH 7.6. Sections were then mounted, air-dried overnight and coverslipped with Permount.

Results
Pathology Immediately After Repeated Exposure to CS₂

Distal axonal swellings in retinogeniculate fibers were observed in the one monkey (113) killed shortly after completion of exposure. (Comparable changes were seen in other axonal populations, including corticospinal and dorsal spinocerebellar fibers and peripheral nerves.) Electron microscopic features of the axonal swellings (not illustrated) included the presence of 10 nm filament accumulations typical of CS₂ exposure in peripheral nerve.1 These swollen axons were present in distal optic tract and terminal axons in anterior LGN, most conspicuously in subpial and juxtavascular locations in the ventromedial half of optic tract (Fig. 1). Swollen axons were not seen in more proximal sections of the optic tract or optic nerve, in retinorecipient regions of superior colliculus or in the geniculostriate pathway. Degenerating myelin profiles were observed in distal optic tract, often abundantly in areas containing few axonal swellings. Subtle astroglial hypertrophy was also present in many areas which contained such active myelin degeneration. Degenerating myelin was identified as far proximally as distal optic nerve, near the chiasm.

Radial sections of retina revealed a moderate loss of ganglion cells centrally; the normal five to six cell deep ganglion cell layer at the margin of the fovea was reduced to a depth of three to four cells, with apparent preservation of the more peripheral ganglion cells. Although few signs of active ganglion cell degeneration were detectable in these sections, occasional ill-defined, dark, shrunken cells were seen within the ganglion cell layer.

Pathology Following a Recovery Period After Repeated CS₂ Exposure

The four monkeys (Nos. 703, 910, 114 and 115) maintained for more than a year after the termina-
Fig. 2. Radial sections through the retina near the fovea, showing marked loss of neurons in the ganglion cell layer (between arrowheads) in CS$_2$-exposed monkey No. 910 (B) compared with control monkey No. 19 (A). Overlying nerve fiber layer is also collapsed. Note absence of abnormalities in vascular profiles (arrows). 1 μM thick resin, Paraphenelene diamine. Scale bar = 50 μm.

tion of exposure also exhibited a loss of ganglion cells. Two monkeys showed permanent visual acuity loss after a single CS$_2$ exposure, while the other two required a second exposure to produce the permanent acuity loss. The normal five to six cell thick retinal ganglion cell layer near the fovea was reduced to three to four cells in monkey No. 115, while in monkeys Nos. 703, 910 and 114, only a few neurons remained at this central location (Fig. 2). Cell counts confirmed that the density of neurons in ganglion cell layer along radial sections was reduced in the central 3.5–4 mm (approximately 15 degrees) of retina (Fig. 3). Neuron densities in peripheral retina of exposed and control monkeys were indistinguishable.

No axonal swellings were detected in the visual pathways of these four monkeys. A sharply circumscribed region of axonal loss and gliosis was seen in lateral (temporal) optic nerve near the globe (Fig. 4) in monkeys Nos. 703, 910 and 114. This region of axonal loss was found progressively more central in distal optic nerve. Axonal loss was concentrated in the dorsolateral sector of the optic tract in monkeys 703, 910 and 114, although the extent of loss varied among monkeys. In monkey No. 115, no gross axonal loss was detectable by inspection, but astroglial hypertrophy was clearly present in proximal regions of the temporal optic nerve and dorsolateral portions of optic tract. In animals Nos. 703 and 910, destruction of axons was so marked that the surrounding nerve tissue collapsed around the involved region.

In both right and left LGNs of monkeys 703, 910 and 114, neuronal atrophy was pronounced only in central portions of parvocellular layers 6, 5 and 4 anteriorly, but in larger portions of layers 6, 5, 4 and 3 more posteriorly. There was also loss of myelinated axons within interlaminar zones in these same areas, along with increased nuclear density: In general, these areas correspond to those receiving projections from central retinal ganglion cells. In monkey 115 parvocellular neuronal atrophy was more subtle than in the other monkeys but also more widespread, involving regions recipient of more peripheral retinal ganglion cells than the other monkeys. No histopathologic changes were identified in superior colliculus or preptectum. There was no obvious loss of axons in dorsal spinocerebellar or corticospinal tracts.
No abnormalities were seen in retinal or cerebral vasculature. Two monkeys in this study had foci of old, gliotic brain necrosis; monkey 114 had virtually symmetrical cavities in both anterior putamens, as well as a microfocus of gliosis in the right substantia nigra, while monkey 115 had a partially gliotic area in right thalamus.

Horseradish Peroxidase Transport Studies

In order to clarify the pattern and extent of retinal ganglion cell loss in monkeys 910, 114 and 115, retinogeniculate projections were traced after intravitreal horseradish peroxidase (HRP) injection. Labelled terminals of preserved retinogeniculate fibers could be seen as branching filamentous processes and as more diffuse, finely granular deposits in the terminal fields of left LGN. The patterns of HRP label in the left LGNs of all three monkeys were spotty and uneven within recipient layers, but some regions had clearly diminished-to-absent HRP label. These areas corresponded closely to the atrophic and gliotic regions of parvocellular layers evident in histologic preparations of both right and left LGNs in each animal. Also present were areas of diminished HRP within magnocellular layers, especially in posterior LGN.

Cytochrome Oxidase Histochemistry

CO histochemistry was used to map regional effects on neuronal activity in the left LGN and striate cortex of monkeys Nos. 910, 114 and 115, and controls. CO activity was most markedly reduced in regions of LGN corresponding to central visual field (Figs. 5, 6). Label was most diminished at the apex of the arch of laminae 6 and 5 anteriorly but over broader regions of these two laminae as well as deeper layers posteriorly. At the most posterior levels, virtually the entire lateral extent of all four parvocellular layers, as well as foci within portions of the magnocellular layers (particularly central portions), showed markedly diminished CO staining. Areas of reduced CO staining in parvocellular left LGN corresponded to regions of diminished HRP label and to histopathologically evident neuronal atrophy in both right and left LGNs in each animal.

In some areas, CO activity was also diminished in layers of left striate cortex (Area 17) that receive projections from the LGN. Reduced cytochrome oxidase reactivity was evident in those regions of Area 17 corresponding to more central visual field representation—and to regions receiving projections from affected areas of LGN (Fig. 7). In such areas, CO staining was virtually absent from layer 4A and markedly diminished in the lower third of layer 4C-beta; while staining in layers 4C-alpha and upper 4C-beta was...
better preserved. CO staining in layer 2–3 “puffs” or “blobs,” and in layer 6 was well-preserved in all cortical regions. In the most markedly affected areas, staining in layers 4C-alpha and upper 4C-beta was also reduced when compared with the intensity of staining in preserved cytochrome oxidase “blobs” or “puffs” in layers 2–3 (Fig. 8). In portions of Area 17 representing the peripheral visual fields, cytochrome oxidase reactivity was better preserved, such that staining in layers 4A or deeper 4C-beta was either normal or only slightly diminished. Neuronal perikarya in Nissl-stained sections of both right and left visual cortex in all animals were preserved in all layers, at all levels.

Discussion

A consistent pattern of neuronal degeneration in the retina and sparing of the retinal vasculature was seen in the carbon disulfide-exposed monkeys of this study. An early effect of carbon disulfide exposure was the development of extensive neurofilamentous swelling in axons of the optic tract (and other fiber tracts). It is not clear how these axonal swellings may be related to the loss of neurons observed with longer survival after exposure. The neurons lost were the
Fig. 7. Reduced cytochrome oxidase reactivity in lateral Area 17 from monkey No. 910 (B), compared with that of a normal monkey (A). Layers 4A and deep 4C-beta in the section from monkey No. 910 clearly have reduced cytochrome oxidase staining compared with control. There is also some loss of cytochrome oxidase staining in upper layer 4C-alpha. Blobs or puffs in layer 2-3 are preserved (arrowheads). 40 \( \mu \text{m} \) section, cytochrome oxidase histochemistry. Scale bar = 500 \( \mu \text{m} \).

Central retinal ganglion cells, especially (but not exclusively) ganglion cells projecting to parvocellular LGN (P-beta or P cells), which appear to play an important role in color vision and acuity. Patterns of gliosis in the retinogeniculate pathway, diminished HRP axonal transport to LGN and altered CO activ-

Fig. 8. Diagrammatic representation of normal cytochrome oxidase reactivity in Area 17 (stippled) in successive coronal levels of posterior cerebral hemisphere, in monkeys Nos. 910, 114 and 115. Black indicates regions of Area 17 with reduced cytochrome oxidase staining (see Fig. 7). Note normal staining in calcarine cortex (representing more peripheral visual fields) in all three animals.
ity in LGN and striate cortex were all consistent with an extensive loss of central retinal ganglion cells. The central retinotopic distribution of effects in LGN were bilaterally symmetrical and appear to be attributable to transsynaptic atrophy and oxidative enzyme (CO) down-regulation (reduced activity) secondary to denervation, rather than to direct toxic injury. The observed ganglion cell degeneration produced substantial and irreversible visual loss in the monkeys of this study. Similar degeneration may explain the permanent visual dysfunction found in many humans exposed to carbon disulfide, including central visual field loss and altered color vision. Despite the marked retinal ganglion cell loss found in these monkeys there was no histopathologic evidence of retinal vascular abnormalities such as those reported in some studies of carbon disulfide-exposed humans.

**Distal Axonopathy**

One reliable effect of experimental CS₂-intoxication has been the induction of distal filamentous axonopathy in peripheral nerves. Neurofilamentous axonopathy in optic nerve fibers has previously been demonstrated in rats exposed to CS₂ as well as in monkeys exposed to acrylamide, or 2,5-hexanediol. The present finding of filamentous swelling in retinogeniculate axons in a CS₂-exposed monkey is further evidence of the sensitivity of the primate (and probably the human) visual system to axonopathic agents. No consistent differences in pattern of ganglion cell loss were detectable between monkeys exposed once and those exposed twice. In addition, there was no obvious relationship between the axonal swellings observed in monkey 113 and the ganglion cell degeneration seen in monkeys that survived the exposure period for several months. The distal axonal swellings seen in optic tract immediately after intoxication were irregular in their distribution, primarily ventromedial in location and concentrated in subpial and perivascular regions. In contrast, axonal loss seen after the prolonged recovery period was most pronounced in the dorsolateral sector of optic tract, in regions corresponding to the location of axons from central retinal ganglion cells. Distal axonal swelling and loss of neurons may reflect two stages of CS₂ effect on retinal ganglion cells; however, the incongruity in the pattern of their respective occurrence suggests that they involve different mechanisms of injury.

The different distributions of axonal swellings seen in the single monkey killed immediately after exposure and the degeneration seen in those killed much later also suggest partial reversibility of the early axonopathy. Otherwise, substantial degeneration would be present in the same regions which contained pronounced swellings in monkeys surviving longer. A similar distinction between axonal swellings and ganglion cell degeneration was observed in acrylamide-induced retinogeniculate effects in primates. In that study, neurofilamentous swellings were distributed throughout the optic tract whereas the later loss of fibers was confined to superior optic tract. In this respect (that the distributions of axonal swellings differ from the distribution of axonal degeneration) carbon disulfide and acrylamide toxicity are similar. Acrylamide and CS₂ also appear related in that both produce degeneration primarily of retinal ganglion cells and that those projecting to parvocellular LGN have a greater vulnerability to both chemicals than those projecting to magnocellular LGN. They seem to differ in that acrylamide has a more pronounced effect on peripheral as well as central P-beta or P cells, while carbon disulfide is more toxic to central P-alpha or M, as well as P-beta or P, retinal ganglion cells.

**Vascular Effects**

The marked visual system injury in CS₂-exposed monkeys occurred in the absence of obvious abnormality of cerebral or retinal blood vessels. Thus, if this damage were due to circulatory alterations, they would have to have been transient. We observed no sign of reduced retinal perfusion (decreased vessel caliber, etc.) during ophthalmoscopy or fluorescein angiography immediately following CS₂ exposure, but we cannot rule out transient vascular disturbances during or even after carbon disulfide exposure. Evidence, both in the present study and previous work, suggests that vascular changes can occur as a result of exposure to carbon disulfide. Localized changes in the brain have been reported in several previous studies, including injury to the basal ganglia in dogs, cats and primates. The focal cavitory lesions in two of the monkeys (Nos. 114 and 115) were consistent with infarcts and they indicate that impaired local perfusion probably occurred in these brain areas, perhaps as a result of carbon disulfide exposure. It is doubtful, however, that such a capricious mechanism could account for the consistently symmetrical, central retinal ganglion cell loss which was found in all exposed monkeys. On the other hand, failure in this study to find retinal vascular effects like those reported in some cases of human exposure, may be due to the short period of exposure of these monkeys relative to that of exposed workers.

In summary, irreversible injury to retinal ganglion cells resulting from chronic exposure to CS₂ was
greatest in the central retina. This pattern of central ganglion cell loss was seen with several months of survival after either one or two exposures, in contrast to the diffuse pattern of distal axonopathy seen initially, suggesting a different mechanism of injury. Although focal necrosis was present in the brains of two monkeys in this study, no permanent histopathologic changes were seen in cerebral or retinal vessels in these or the other monkeys. The ganglion cell loss reported here occurred in the absence of significant arteriosclerosis or of retinal aneurysm formation and probably reflects a form of direct toxic effect on these cells. Neuron size, metabolic activity, neurotransmitter or other characteristics of retinal ganglion cells may underly their vulnerability to carbon disulfide.

Key words: retinal ganglion cells, visual toxicity, neuronal degeneration axonopathy, carbon disulfide, macaque monkey

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References