Lesions have been described in the optic nerves and corpora callosa of the rat following the administration of sodium cyanide. There is a tendency for the lesions to involve the retrobulbar zone of the optic nerve, but the retina is not affected. The distribution of lesions may be determined by variations in the vascular anatomy. Previous studies have not identified the cell or cell component within the optic nerve which is the initial site of damage. We have attempted to ascertain this by examining the fine structural changes that occur in the optic nerve and retina of the rat after intoxication with sodium cyanide.

Materials and methods

Adult, male, albino, Charles River rats were used exclusively. Their initial weight ranged from 260 to 480 grams and they gained weight during the experiment. Each rat was given several small intraperitoneal injections of a solution of sodium cyanide sufficient to keep the rat in coma for 225 to 260 minutes. Rats were killed at 2 days, 1, 2, 3, 4, and 6 weeks. The retinas and the optic nerves were excised after enucleating the eye during craniotomy, and they were fixed in a 4 per cent glutaraldehyde solution for 20 minutes. The retina was obtained from the posterior pole. Optic nerve was sampled at two sites—3 to 4 mm. behind the globe and in the pre-chiasmal area. The tissue was divided into small pieces (measuring not more than 0.3 mm. cubed) and postfixed in 1 per cent osmium tetroxide. These fragments were dehydrated in a series of ethyl alcohols and were embedded in an epoxy resin. Five-tenths micron thick sections were stained with toluidine blue and studied with the light microscope. Ultrathin sections were stained with uranyl acetate and lead citrate and were examined with the electron microscope.
Fig. 1. The optic nerve of a normal rat. Axons are of various sizes and regularly myelinated. They are grouped into bundles separated by astrocytic processes. Higher magnification reveals that axons contain regularly spaced microtubules and scanty smooth endoplasmic reticulum. Small cytoplasmic ends of the oligodendroglia cells are seen outside of the myelin sheath (arrows). Similar mesoaxons are seen inside, but the axon is closely attached to the myelin sheath. Left, x30,000; right, x9,000.

Observations

Normal optic nerve of the rat. The optic nerve of the normal rat consists of numerous myelinated axones measuring from 0.5 to 2 μ in diameter. They are grouped into bundles separated by the cell processes of astrocytes (Fig. 1). The axoplasm is electron lucent and contains regularly spaced microtubules, mitochondria, neurofilaments, and occasional smooth endoplasmic reticulum. The myelin sheath is formed by a double membrane wrapped in a spiral around each axone to form an average of five to seven layers. Fixation artifacts are commonly found in the myelin sheath. The thin mesoaxonal tissue lies between the axone membrane and the myelin sheath, and a small triangular segment of tissue, the outer mesaxon, is regularly found at the outer edge of the myelin sheath. Although blood capillaries and a few collagen fibers are found in the optic nerve, septae are not present. The astrocytes extend processes in a stellate pattern and their fine ramifications appear to fill in the spaces between many axons. Gap junctions and desmosomes are encountered frequently between these processes. The cytoplasm of the astrocyte contains an electron-lucent matrix and numerous fine fibrils. The cytoplasm of the oligodendrocyte contains abundant rough endoplasmic reticulum and is dark in comparison to that of the astrocytes. The fine processes of this cell appear to form the outer mesoaxons which are the outer limit of the myelin sheath.

Pathologic changes in the optic nerve. As in the earlier report, severe pathologic changes were found in the retrobulbar zone. The pathologic changes described below apply primarily to this retrobulbar portion of the nerve. Sections from the prechiasmal zone showed only mild changes. The optic nerve was grossly edematous in the rats killed at two days and at one week. Astrocytes were enlarged and clumping of myeline was found as early as the second day (Fig. 2). Electron microscopy revealed considerable nerve fiber damage even after this short interval after intoxication. Some fibers were irregularly shaped and exhibited enlarged inner mesoaxons (Fig. 3). Axons were slightly shrunken but their microtubules appeared to be normal. Pathologic myelin sheaths were also occasionally present at this stage. Whirled masses of myelin membranes from which axons had disappeared were found occasionally (Fig. 3). They were of various sizes (0.5 to 5 μ) and were found frequently in the vicinity of oligodendroglial cells. The oligodendroglial cells were more hyperchromatic than normal and their cytoplasm contained enlarged rough endoplasmic reticulum and swollen mitochondria. No other glial reaction or phagocytosis was found within the first week.

Pathologic changes were prominent after two
Fig. 2. Forty-eight hours after the intoxication astrocytes are markedly swollen. Oligodendroglia cells are markedly electron dense. Toluidine blue stain, ×500.

Fig. 3 One week after the intoxication. Irregular whirls of myelin are seen in the vicinity of an oligodendroglia cell, which has enlarged rough endoplasmic reticulum (asterisk). The nerve fibers have enlarged mesaxon. ×10,000.
Fig. 4. Three weeks after the intoxication. Myelin masses are found abundantly. Many axons are markedly swollen. Toluidine blue stain, ×500.

Fig. 5. Four weeks after the intoxication. Large intercellular spaces (arrows) are present. Axons are shrunken and enlarged mesoaxons are apparent. A large myelin mass from which the axon has disappeared is seen in the center. The blood capillary appears to be normal. ×8,500.
Fig. 6. Four weeks after the intoxication. There are numerous myelin masses. There is an electron-dense necrotic axon present (asterisk). \( \times 11,000 \).

Fig. 7. Four weeks after the intoxication. Nerve fibers in the area away from the degenerating focus show shrinkage of axons and enlargement of mesaxon (arrows). The cytoplasm of an oligodendroglia cell contains rich ribosomes and enlarged rough endoplasmic reticulum. \( \times 30,000 \).
Fig. 8. Four weeks after the intoxication. Many axons are markedly swollen and have no microtubules. Some axons are necrotic (asterisk). Denuded axons are seen occasionally (arrows). x11,000.

Fig. 9. Six weeks after the intoxication. The nerve fibers are markedly degenerated. The area is occupied by numerous myelin masses. Toluidine blue stain, x500.

weeks. A great number of the nerve fibers were in a degenerating state (Fig. 4). Astrocytes were swollen, and large intercellular spaces were seen. Severely damaged nerve fibers were abundant (Fig. 5). Damaged axons were shrunken or vacuolated and had sparse microtubules. Many nerve fibers showed enlarged mesaxons. Large whirled myelin sheaths, from which axon processes had disappeared, and small fragments of myelin were numerous. Some axons appeared to have been...
Fig. 10. Six weeks after intoxication. Normal nerve fibers are not seen in this area. Myelin whirls and fragments of myelin sheaths are scattered in the glia cells. ×11,000.

Fig. 11. Six weeks after the intoxication. Ganglion cell layer of the retina shows a normal ganglion cell (black asterisk) and a markedly shrunken ganglion cell with the pyknotic nucleus (white asterisk). The Muller cell contains an increased number of glycogen particles and enlarged mitochondria. ×12,000.
come electron-dense, necrotic masses (Fig. 6). No pathologic changes were seen in capillaries. After three weeks, there was an increase in the number of degenerating nerve fibers, and they were spread over a larger area. Enlargement of the mesaxon appeared in relatively intact optic nerves during this period (Fig. 7). Oligodendroglial cells showed an enlarged rough endoplasmic reticulum. No phagocytic activity was demonstrated.

Degeneration of the nerve fibers was conspicuous in the fourth and fifth week. Few normal nerve fibers were in evidence in the retrobulbar zone. There were numerous large swollen nerve fibers, necrotic axons, and myelin masses. Clusters of totally demyelinated axons were found frequently in this area (Fig. 8).

The optic nerve was grossly atrophic at six weeks. The retrobulbar zone of the nerve was small and the sections revealed marked degeneration (Fig. 9). The number of nerve fibers was markedly reduced and the pathologic area was occupied by glial cells. The remaining axons were markedly swollen or necrotic. Myelin whorls of various sizes were scattered in the degenerated area (Fig. 10).

The posterior retinas of the experimental rats were examined by both light and electron microscopy at various stages following the injection of sodium cyanide. Light microscopic examinations revealed no appreciable changes at any stage of the experiment. However, electron microscopy revealed considerable changes in the retinas examined at the four week interval after intoxication. The most striking change was shrinkage of some ganglion cells. These cells were pyknotic nuclei and atrophied, electron-dense cytoplasm (Fig. 11). These cells were occasionally found among normal ganglion cells. Some cells may have already degenerated because the area occupied by the Muller's cells was found to be larger than was seen in normal eyes. The surviving ganglion cells appeared to be normal in terms of their cytoplasmic constituents except for the presence of swollen mitochondria. The nerve fibers were irregular in shape, apparently due to localized swelling. The swollen portion of the nerve fibers contained markedly swollen mitochondria. Microtubules were absent in the swollen nerve. The cytoplasm of the Muller's cells in the inner layers of the retina was expanded. The enlarged cytoplasm contained increased amounts of glycogen particles and markedly swollen mitochondria. The swollen mitochondria often measured 1 μ in diameter and showed rich cristae (Fig. 12).

Discussion

The present study has demonstrated the cytologic details of the lesions in the rat optic nerve caused by cyanide poisoning. Swelling of the astrocytes, degeneration of
the nerve fibers, and reactive changes in oligodendroglial cells were seen as early as 48 hours after the intoxication. The pathologic process appears to be slowly progressive. The progress of the degeneration seemed to accelerate after the third week, and the optic nerve became severely atrophic by the sixth week. As shown in the earlier light microscopy study, the pathologic changes occur primarily in the retrobulbar zone of the nerve.

Actual degeneration of the astrocytes was not found in any stage of the experiment. There was also surprisingly little inflammatory reaction to, and phagocytosis of, the neuronal debris. Why this is, is not clear to us, but in the earlier experimental study, a marked microglial reaction was documented at the site of lesions in some animals.2, 3 Shrinkage of axons appeared in many nerve fibers at all stages of the experiment. Swelling and vacuolation, which seemed to lead to ultimate disappearance of the axon were found also. In some cases there was an alteration in appearance of the cytoplasm of axons such that they became quite electron dense. It is noteworthy that the myelin sheaths of these pathologic axons appeared to be intact. Fragmentation and degeneration of myelin seemed to occur secondarily. The primarily axonal origin of the neural damage in experimental cyanide encephalopathy elsewhere in the brain has previously been demonstrated electron microscopically by others.4 The histologic appearance of demyelination that is seen in the late stages is the result of the initial loss of axons. A few axons without myelin sheaths are occasionally found at a late stage of the intoxication, but their number is small.

All of the blood vessels found in and around the pathologic lesions were patent, and their endothelial cells seemed to be normal. To a small extent the ganglion cells and nerve fibers of the retina showed pathologic changes. However, no acutely necrotizing process was found within the retina. The pathologic changes in the ganglion cells may be considered as atrophy secondary to the axonal lesion in the nerve rather than a primary degeneration. It is of interest that we found markedly enlarged mitochondria in the Muller's cells. The increase in mitochondrial volume might represent an attempt to compensate for the inhibitory effect of cyanide on metabolism. Curiously, the enlarged mitochondria were seen only in the Muller cells and not in the neural cells of the retina.

We would suggest that the sequence of events in the optic nerve of the rat after cyanide intoxication is as follows. The initial dysfunction is in the metabolism of the axons due to the action of cyanide on respiratory enzymes. Relative lack of capillaries in the retrobulbar zone accentuates the toxic effects in that area. Simultaneously, the astrocytes responsible for the maintenance of axonal metabolism hypertrophy in a vain effort to rectify the situation. Axonal degeneration then occurs with ultimate loss of axons, secondary demyelination, and gliosis.

The authors wish to thank Miss Marjorie Parmenter and Mr. Denifield Player for their skilled assistance in this investigation.

The authors wish to dedicate this work to Dr. David G. Cogan.

REFERENCES