Methyl Alcohol Poisoning

IV. Alterations of the Morphological Findings of the Retina and Optic Nerve

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• The ocular morphological findings of three methyl alcohol-intoxicated rhesus monkeys with optic disc swelling was investigated with light and electron microscopy in conjunction with intravascular horse radish peroxidase. Alterations observed in the optic nerve head were confined to the axons and consisted of swelling and clustering of the mitochondria, disruption of the neurotubules, the formation of vesicles, and enlargement of the axon segments in the prelaminar region. Swelling of the oligodendroglial cytoplasm in contact with the axons and of the astrocytes was seen in the retrolaminar optic nerve and the intraorbital optic nerve. Alterations were not observed in the retina. It is hypothesized that the alterations in the axons are the result of disrupted axoplasmic flow. Possible mechanisms relating methyl alcohol intoxication to disruption of axoplasmic flow are discussed.

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The alterations of the ocular morphological findings produced by methyl alcohol ingestion has been the subject of several investigations during the better part of the last 100 years. The light microscopic observations reported in the literature are variable, but in general support the view that retinal ganglion cell degeneration is the primary lesion[1-3]; and that when swelling and demyelination are present in the optic nerve, it is either secondary to the degeneration of the retinal ganglion cells[1,2] or it is a simultaneous and independent alteration.[3] This concept implies that the optic atrophy, which often follows methyl alcohol poisoning, is secondary to injury of the retinal ganglion cell.

The development,[4] refinement,[5] and documentation[6] of a consistent model of methyl alcohol poisoning in the rhesus monkey provided the opportunity to investigate the alterations of its ocular morphological findings with the electron microscope. By utilizing an experimental animal, meaningful ultrastructural observations were possible since problems caused by artifacts could be controlled. The observations of this study showed that the primary sites of ocular injury produced by methyl alcohol intoxication were the optic nerve head and the intraorbital portion of the optic nerve rather than the retinal ganglion cells. Further, it demonstrated that the optic disc edema described by Hayreh et al[6] was the result of intracellular rather than extracellular swelling. Finally, a hypothesis for the morphological alterations based on the concept of disruption of anterograde axoplasmic flow is explored.

MATERIALS AND METHODS

Light and electron microscopic studies were done on the retina, the optic nerve heads, and the optic nerves of two control and three methyl alcohol-poisoned rhesus monkeys. A nonlethal, acidotic state of methyl alcohol intoxication was achieved with periodic oral doses of methyl alcohol at intervals determined by the appropriate arterial blood levels of pH, Po_2, Pco_2, HCO_3, formate, and methyl alcohol as described by Martin-Amat et al.[5]

Pupillary responses to light were monitored before and during the course of intoxication. Fundus changes were periodically documented with stereoscopic color fundus photography and fluorescein angiography as detailed by Hayreh et al.[6] In addition, the CSF pressure and formate level were measured in two of the animals just prior to killing.

Horse radish peroxidase, an electron-dense protein tracer,[7,8] was injected intravenously two hours before killing (500 mg/kg of body weight). Tissue fixation was accomplished by the intracardiac perfusion of one liter of 1% formaldehyde-1.25% glutaraldehyde in a 0.08M sodium cacodylate buffer at pH 7.4, followed by 0.5 liters of 4% formaldehyde-5% glutaraldehyde in the same buffer.[8,9] The eyes were then enucleated with a segment of optic nerve, dissected, and immersed in the more concentrated fixative for 24 hours.

The intraorbital optic nerves, the optic nerve heads, and representative pieces of the retina were sectioned at 50 μ with a tissue sectioner, and incubated at room temperature in a solution of 5 mg of 3, 3′ diaminobenzidine tetrahydrochloride, 10 ml of 0.05M TRIS buffer at pH 7.6, and 1% H_2O_2 for 30 to 40 minutes.[7,8] The tissue sections were then postfixed in 1% osmium tetroxide, stained en bloc with 0.5% uranyl acetate,[8,10,11] dehydrated, and embedded in epoxy resin (Epon 812). Thick sections of 2 μ were mounted on glass microscope slides and stained with toluidine blue or Richardson stain for light microscopic examination. Thin sections of appropriate tissue blocks were cut on an ultramicro-

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Summary of Biochemistry and Ophthalmoscopy

Animal No.	Time, hr	Ophthalmoscopy	CSF: Pressure, mm H_2O	CSF: Formate, mEq/Liter	Blood: Formate, mEq/Liter	Blood: HCO_3, mEq/Liter	Blood: pH	Blood: CH_3OH, mg/100 ml
R1	27	Normal	...	...	11.5	20	7.26	100
	48*	Marked swelling of the optic disc	40	13.9	12.9	17	7.13	139
R5	27	Normal	...	...	10.7	23	7.36	128
	69*	Swelling of the optic disc with blurred disc margins	53	7.9	11.2	21	7.32	116
R6	50	Normal	...	...	5.2	13	7.34	40
	171*	Optic disc hyperemia with swelling	...	...	13.1	15	7.27	81

*Killed.

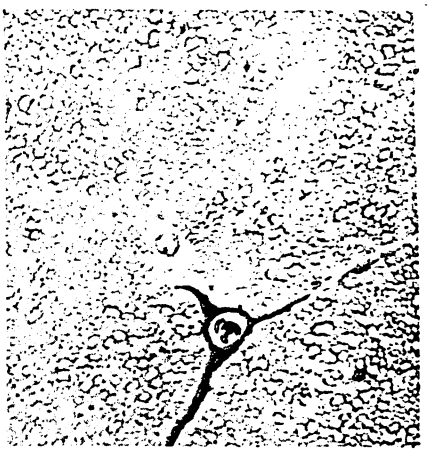


Fig 1.—Transverse section of intraorbital optic nerve of control animal with myelinated axons. Fibrovascular septum is present in lower field (×1,800).

Fig 2.—Longitudinal section of optic disc of control animal at level of laminar and retrolaminar regions. Note addition of myelin sheaths in retrolaminar region (×1,800).

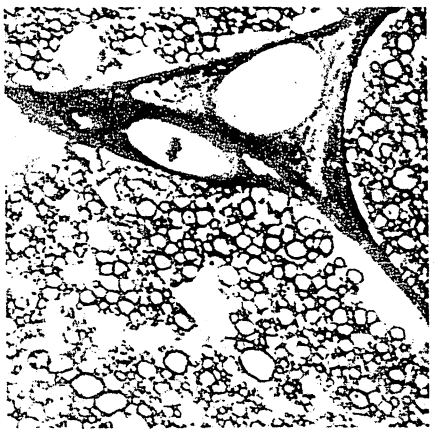


Fig 3.—Transverse section of intraorbital optic nerve of methyl alcohol-intoxicated animal showing clear spaces within myelin sheaths, between them, and adjacent to collagen vascular channels (×1,800).

Fig 4.—Longitudinal section of optic nerve head of methyl alcohol-intoxicated animal at level of laminar and retrolaminar regions. Most of clear spaces are bounded by myelin (×1,800).

tome, stained with lead citrate, and examined with an electron microscope.

RESULTS

Biochemistry and Ophthalmoscopy

Results from the biochemical measurements of Martin-Amat et al[5] and the ophthalmoscopic and the CSF pressure observations of Hayreh et al[6] are summarized in the Table. Two animals (R1 and R5) attained blood formate levels of 11 to 14 mEq/liter within the first 24 hours of intoxication and were observed to have swelling of the optic disc 48 hours after the first administration of methyl alcohol. The third animal (R6) maintained lower levels of blood formate (2 to 6 mEq/liter) during the first 72 hours of intoxication, but when swelling of the optic disc was observed 171 hours after the first methyl alcohol dose, the blood formate level was 13.1 mEq/liter.

Morphological Findings

Light micrographs of the intraorbital optic nerve (Fig 1) and the retroaminar optic nerve (Fig 2) from the control animals revealed normal myelination of the axons, intact astrocytes, and no discernible extracellular space. In contrast, clear spaces of variable shapes were observed within the myelin sheaths of these same regions in each of the three animals intoxicated with methyl alcohol (Fig 3 and 4). Ultrastructurally, these spaces either displaced neurofilaments within axons or compressed the axon against the inner surface of the myelin sheath (Fig 5). The compressed axons were separated from these spaces by a narrow band of extracellular space and a second unit membrane, and were a part of the cytoplasmic extension of an oligodendroglial cell (Fig 6).

Other morphological alterations in the intraorbital optic nerve included a decrease in the cytoplasmic density of many glial cells and their processes, particularly the foot processes adjacent to collagen vascular channels (Fig 3). Other clear spaces of uncertain cellular origin were observed in the optic nerve parenchyma between myelinated sheaths (Fig 3). These spaces

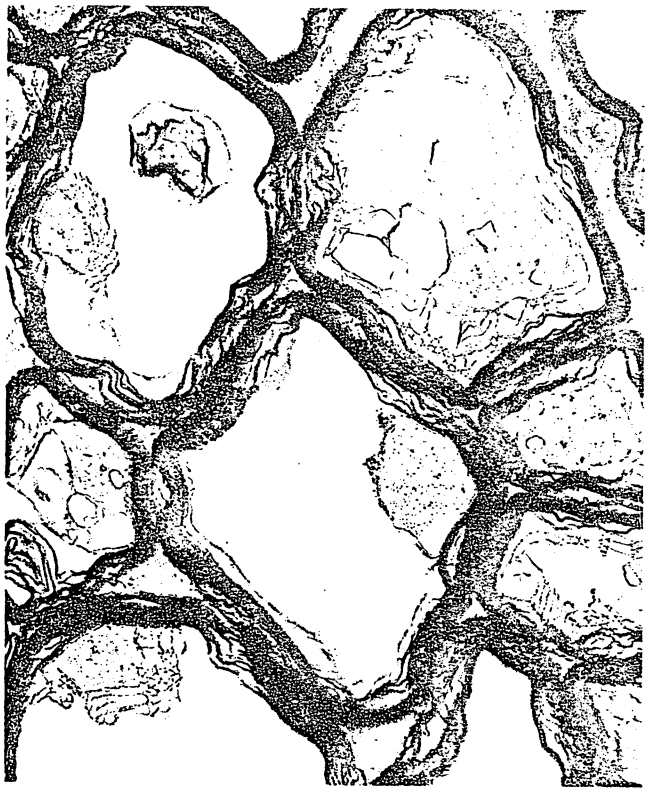


Fig 5.—Transverse section of optic nerve of methyl alcohol-intoxicated animal. Axons are compressed within myelin sheaths. Honeycomb pattern is caused by unraveling of myelin (×14,400).

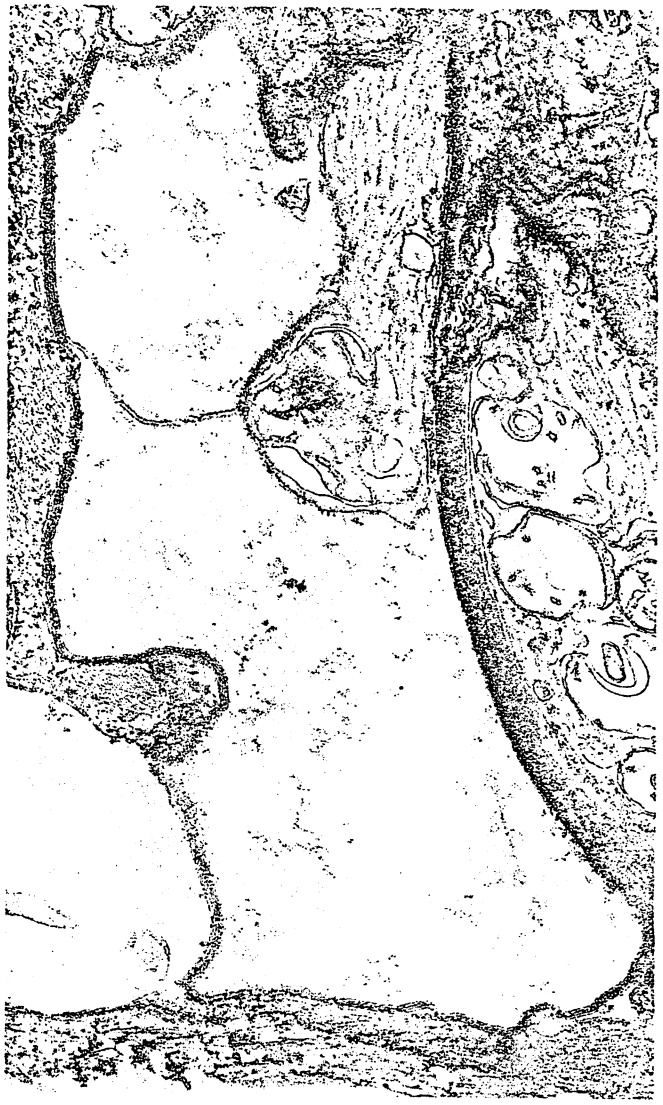


Fig 6.—Swollen cytoplasmic extension of oligodendroglial cell contacting axon in postlaminar optic disc (×18,400).

contained swollen mitochondria, variable amounts of granular material, and were bounded by a unit membrane that separated them from other structures, leaving only a small amount of extracellular space (Fig 7).

Intraaxonal mitochondrial alterations were a prominent and significant finding at the ultrastructural level within the retrolaminar optic nerve, and the laminar and the prelaminar optic nerve head (Fig 8 through 10). The mitochondria were enlarged, and there was a reduction in the number of cristae together with a corresponding loss of dense matrix material. The numbers of mitochondria clustered within one axon in cross section were increased. Associated with the mitochondrial alterations were the absence of recognizable neurotubules or neurofilaments, the appearance of numerous vesicular bodies, and an increased density of amorphous proteins. By comparison, the ultrastructure of the axons in the optic nerve heads of the control animals was unaltered. The mitchondria were not swollen or clustered, the neurotubules and neurofilaments were evenly spaced, and vesicular bodies were not seen (Fig 11).

A second type of axonal alteration was seen in the prelaminar optic nerve head and the surface nerve fiber layer in animals R1 and R6 (Fig 12). It consisted of a decreased density of the cytoplasm, small aggregations of granular material, a few scattered relatively intact mitochondria, and fragmentation of the unit membranes that separated these structures from the adjacent dense astrocytic processes and relatively intact axons. These altered processes represented the remains of enlarged axons based on their anatomical relationship with surrounding recognizable structures and by comparing their location to similar regions from a control animal. With the light microscope, these relatively electronlucent regions were seen to be translucent spaces of variable sizes and shapes (Fig 13). They were more numerous in the surface nerve fiber layer and extended peripherally into the nerve fiber layer of the peripapillary retina. But no other alterations were observed in this region of the retina.

The macula and random sections of peripheral retina from the methyl alcohol-poisoned animals showed only those morphological alterations also observed in the control animals (Fig

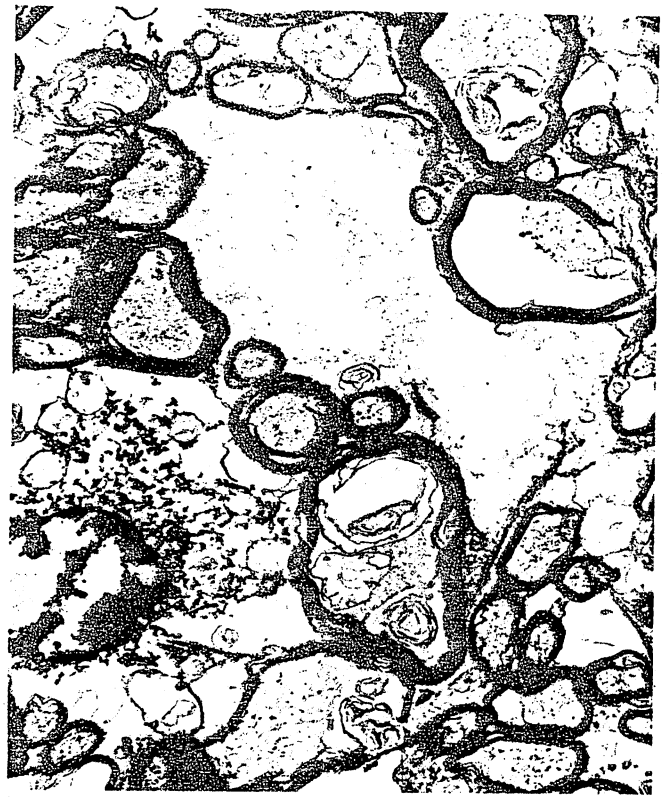


Fig 7.—Alterations of this glial cell in optic nerve include clumping of nuclear chromatin, mitochondrial swelling, and dispersion of cytoplasmic proteins (×7,200).

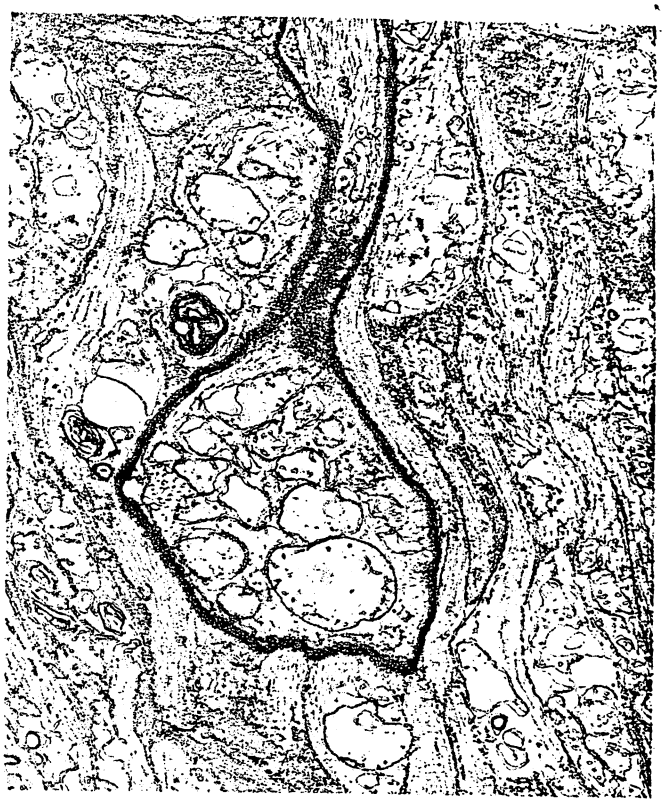


Fig 8.—Myelinated and unmyelinated axons in retrolaminar optic disc showing clusters of swollen mitochondria (×9,600).

Fig 9.—Altered axons in laminar optic nerve head showing formation of vesicles. Black material in lamina cribrosa is peroxidase (×9,600).

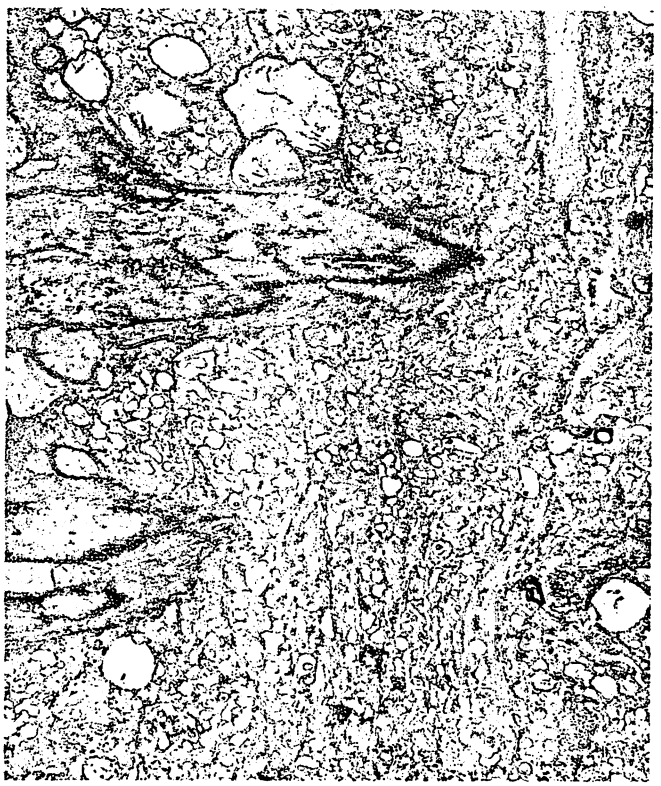
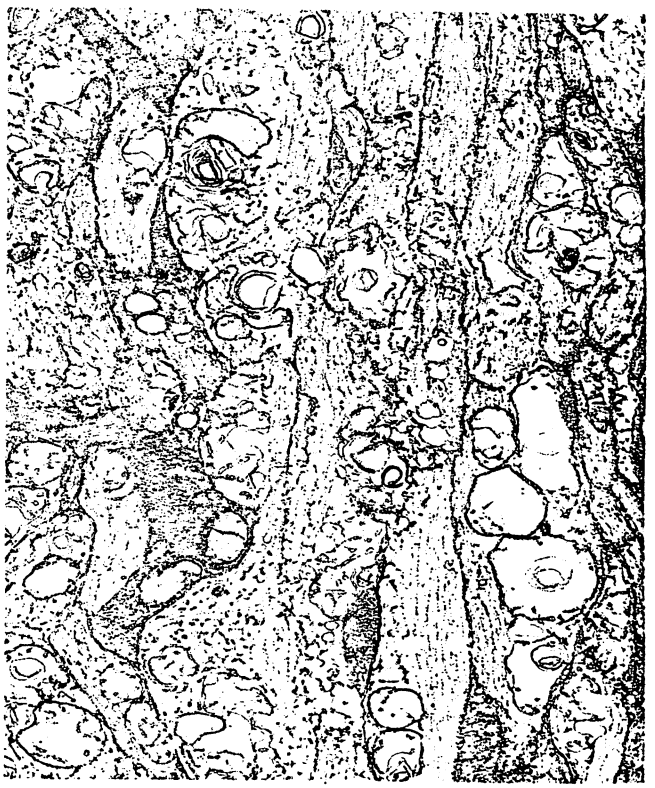


Fig 10.—Axonal alterations in prelaminar optic nerve head include clusters of swollen mitochondria and disruption of some neurotubules (×9,600).



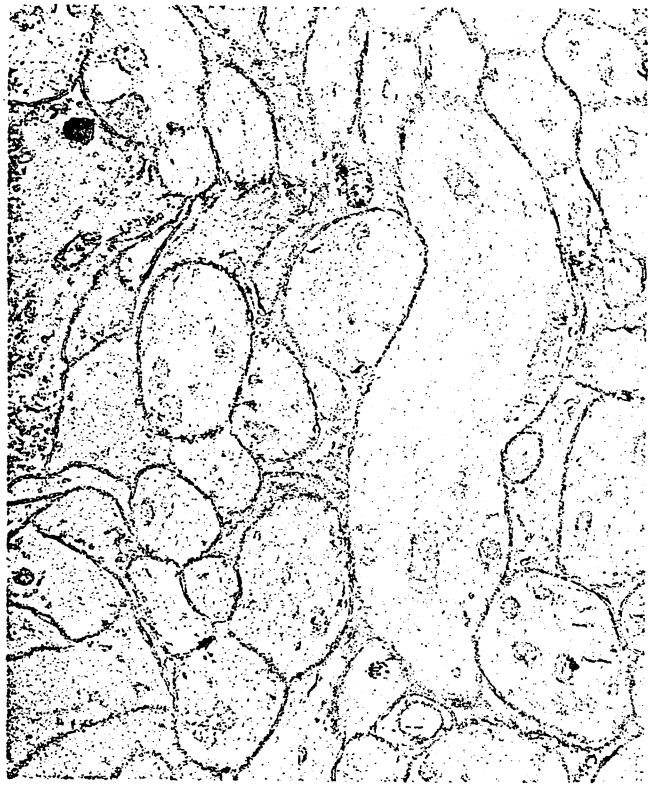


Fig 11.—Longitudinal section of optic nerve head of control animal showing well-preserved mitochondria and neurotubules (×9,800).

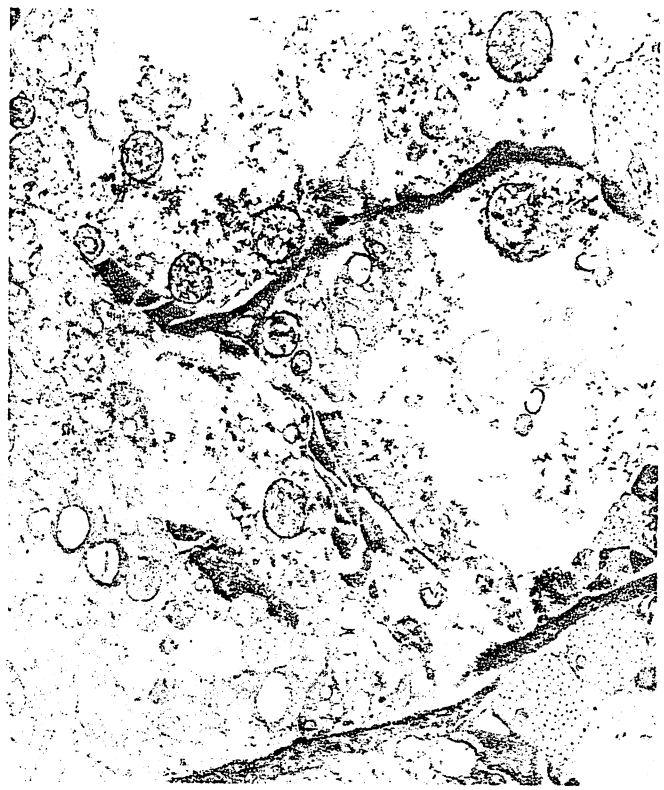


Fig 12.—Swollen axons in prelaminar optic nerve head near the inner limiting membrane. There is swelling of mitochondria, dispersion of cytoplasmic contents, and fragmentation of cytoplasmic membranes. Note dense processes of astrocytes (×10,000).

14 and 15). These observations included the retinal ganglion cell and nerve fiber layers. The ganglion cells in all regions of examined retina had a centrally placed nucleus with a prominent nucleolus, and a moderate amount of cytoplasm without peripheral displacement of Nissl substance. Ultrastructurally, there was minimal swelling of the mitochondria and loss of cristae, but this finding was also present in the control tissue.

The diffusion of horse radish peroxidase into the optic nerve heads of the methyl alcohol-intoxicated animals was the same as that observed in the control animals. The source of horse radish peroxidase leakage was the peripapillary choriocapillaris. It then diffused between the astrocytic processes, which form the border tissue of Jacoby, into the extracellular spaces of the optic nerve head.

COMMENT

The morphological alterations seen in the optic nerve and optic nerve head in methyl alcohol-poisoned rhesus monkeys[4,5] can be separated into two categories—alterations within axons and alterations of glial cells. Alterations seen in axons include mitochondrial swelling and clustering, neurotubular disruption, the formation of vesicles, and increased density of amorphous proteins, and axonal enlargement. Glial cell alterations include astrocytic swelling and swelling of the oligodendroglial cytoplasm in contact with the optic nerve axon.

An explanation that accounts for the observed intraaxonal alterations is based on the process of anterograde axoplasmic flow and the alterations that occur when the flow is interrupted. Simply defined, anterograde axoplasmic flow is a metabolically active process that transports proteins,[12-14] mitochondria,[15] and neurotubules[16] from their site of synthesis in the neuronal perikaryon, along the axon, to its synaptic termination. The different cytoplasmic components have different flow rates,[13] the mitochondria and neurotubules comprising the slow component, and the proteins making up the fast component. Disruption of anterograde axoplasmic flow results in the damming back of the axoplasm proximal to the disruption. This produces clustering of mitochondria,[15] seen as an increase in the numbers of mitochondria per cross section of axon, and fragmentation of the neurotubules and neurofilaments.[14] Both of these findings were observed in the optic nerve heads of the methyl alcohol-poisoned rhesus monkeys. It is therefore reasonable to suppose that intoxication with methyl alcohol causes disruption of axoplasmic flow somewhere in the retrolaminar optic nerve or the laminar region of the optic nerve head. If the flow of axoplasm continues, it will build-up and cause swelling of the proximal axon segment. Compared to observations made in the control tissue, many of the altered axons in the optic nerve heads of the methyl alcohol-poisoned animals appeared to be enlarged,

Fig 13.—Longitudinal section of prelaminar optic nerve head of methyl alcohol-intoxicated animal. Clear spaces are more numerous near inner limiting membrane (×600).

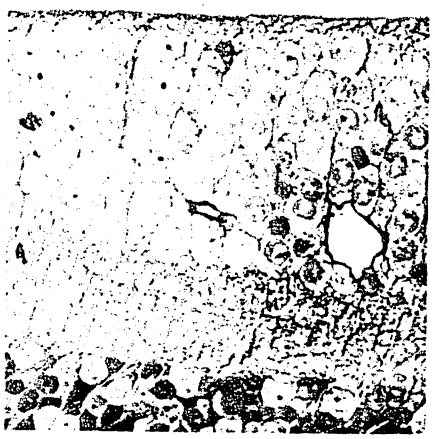


Fig 14.—Section of control retina in parafoveal region of macula showing well-preserved retinal ganglion cells (×1,800).

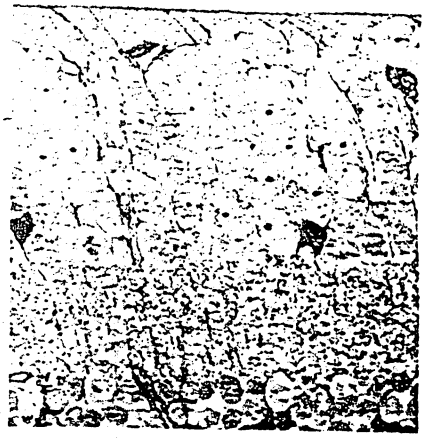


Fig 15.—Section of methyl alcohol-intoxicated retina in parafoveal region of macula showing unaltered retinal ganglion cells (×1,800).

particularly in the anterior region of the prelaminar portion (Fig 12).

There are several causes of disrupted axoplasmic flow, including mechanical obstruction of the axon,[14,15] and several chemical agents such as cyanide,[17-20] which is a known inhibitor of cytochrome oxidase,[21] and colchicine, which causes depolymerization of the neurotubules.[12,22] Therefore, it is reasonable to hypothesize that methyl alcohol or more likely formate, which is a metabolite of methyl alcohol[5] and an inhibitor of cytochrome oxidase,[23,24] disrupts the flow of axoplasm. A possible mechanism of disruption is as shown in Fig 16.

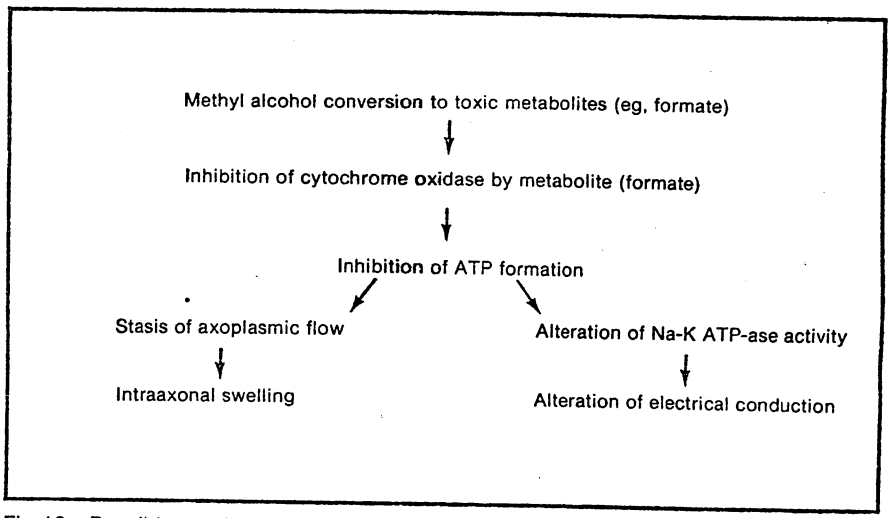


Fig 16.—Possible mechanism of metabolic disruption caused by methyl alcohol.

That metabolic dysfunction occurs within mitochondria is indirectly supported by the morphological finding of generalized swelling of intraaxonal mitochondria.

As previously stated, the disruption of axoplasmic flow in the axon of the retinal ganglion cell appears to be localized to one part of the axon rather than interfering with axoplasmic flow along its entire length. If it were a generalized process, intraaxonal alterations should have also been observed in the retinal nerve fiber layer, which was not the case. Furthermore, swelling of the axons in the laminar and prelaminar optic nerve head could not be due to the build-up of axoplasm since axoplasm must be actively transported to the nerve head. The localization of disrupted axoplasmic flow to the retrolaminar optic nerve must be explained. If formate is to cause disruption of the axoplasmic flow in the retrolaminar optic nerve, it must be able to come in contact with the axons in this region. This and other studies[25-27] have shown that horse radish peroxidase, a protein about the size of albumin,[8] when injected into the circulatory system is able to diffuse out of the fenestrated capillaries of the choriocapillaris into the extracellular spaces of the optic nerve head and retrolaminar optic nerve. Formate, being a much smaller molecule than horse radish peroxidase, should be able to diffuse into the optic nerve head and retrolaminar optic nerve along the same route. It has also been shown that the CSF communicates freely between the subarachnoid spaces of the brain and the optic nerve.[28] Horse radish peroxidase, injected into the lateral cerebral ventricles, can be traced through the subarachnoid spaces of the optic nerve sheath, between the processes of the pia, and into the extracellular spaces of the optic nerve as far anteriorly as the retrolaminar optic nerve and the optic nerve head. Therefore, if the formate found in the CSF at the level of the cisterna magna is in equilibrium with the rest of the CSF system, further support is added to the premise that the axons in the retrolaminar optic nerve head are exposed to formate. However, the finding of formate in the CSF indicates that

formate is apparently able to diffuse across the blood-brain barrier. Because the blood-optic nerve and blood-retinal barriers to horse radish peroxidase[28-31] are similar to the blood-brain barrier to horse radish peroxidase,[32,33] it is likely that the lack of a barrier to vascular formate in the brain would also hold true in the optic nerve and the retina. Thus it is probable that the parenchyma of the retina is exposed to formate. But the retinal morphological findings, unlike those of the optic nerve head, are unaltered even after seven days of methyl alcohol intoxication. This means that formate is either selectively excluded from the retina, or that because of certain differences, the retrolaminar optic nerve is more susceptible than the retina to the levels of formate achieved in this study. A major anatomical difference is the addition of the oligodendrocyte that myelinates the axons beginning in the retrolaminar optic nerve. The significance of this difference is supported by the observation of swelling of the oligodendroglial cytoplasm between the axons and the inner surface of the myelin sheaths beginning in the retrolaminar optic nerve. The swelling may then mechanically compress the axon within the myelin sheath causing disruption of the flow of axoplasm, either by itself or in conjunction with altered metabolism of the axon. This anatomical difference is supplemented by the finding that cytochrome oxidase activity is lower in white matter than in gray matter.[34] This can be interpreted to mean that the myelinated part of the optic nerve is more susceptible to metabolic inhibition by formate than the retina, especially considering the likelihood that the lower levels of cytochrome oxidase indicates a lower metabolic reserve.

Whatever the cause of interrupted axoplasmic flow, the retinal ganglion cell body must remain functionally intact if the build-up of axoplasm proximal to the disruption is to occur. This is supported in this study by the finding of intact ganglion cells at both the light and the electron microscopic levels. Because of the differences in the species of experimental animals, and in the techniques of intoxication and fixation, comparisons of the observations of this study with the observations of past studies[1-3] are not easily made. However, the finding of unaltered retinal ganglion cells in this model of methyl alcohol intoxication is further supported by the ophthalmoscopic and fluorescein angiographic findings of Hayreh et al.

Though further studies directed towards determination of axoplasmic flow are needed to establish the hypothesis of axoplasmic flow disruption and to further delineate the mechanism through which methyl alcohol produces ocular toxicity, this study points to the optic nerve head and not the retina as the primary site of injury in methyl alcohol poisoning.

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