The pathology of the optic nerve resulting from experimental acute glaucoma was studied by electron microscopy in owl monkeys (Aotus trivirgatus). Two distinct stages in the evolution of the pathological changes were recognized. The first stage consisted of widespread axonal changes with or without focal areas of necrosis. The axons showed severe hydropic swellings and/or reactive axonal enlargements filled with mitochondria, dense bodies, vesicles, and membranous whorls. The hydropic degeneration of the axons accounted for the vacuolar changes seen by light microscopy in severely affected nerves. No enlargement of extracellular spaces and no penetration of vitreous could be demonstrated at this stage. The second phase, developing several days after onset of glaucoma, began with the cystic transformation of necrotic tissue beneath the internal limiting membrane, causing its dissolution or rupture in focal areas. Vitreous, together with injected electron-dense markers (Thorotrast or India ink), was forced through these gaps into the damaged nerve, producing cavernous extracellular spaces. The degenerated axons and other debris were removed by phagocytes. The origin of at least some of these cells from hematogenous monocytes is discussed and illustrated.

The pathogenesis of Schnabel's cavernous degeneration of the optic nerve in glaucoma has recently been studied experimentally in the owl monkey. These studies have presented evidence of a marked reduction of blood flow in the optic nerve head when there is an acute rise in intraocular pressure. Kalvin and co-workers concluded that this reduction in circulation was responsible for the cavernous degeneration. Zimmerman and co-workers, while in essential agreement that the ischemia was probably the most significant pathogenetic factor, pointed out that there are noteworthy differences in the histopathologic features of an ischemic infarct and cavernous degeneration. In the latter, a microglial and astrocytic response is negligible, and the cavernous spaces are filled with hyaluronic acid. In the infarct, the macrophagic and glial reactions are characteristic, while an accumulation of hyaluronic acid is not observed. It was postulated that Schnabel's cavernous degeneration is a special type of infarct resulting from acute ischemia in which hyaluronic acid from the vitreous is forced
backward into the necrotic tissues of the nerve head. It was suggested that perhaps the hyaluronic acid has an inhibitory effect upon the cellular response. 0

In the previous light microscopic studies, 0 severe hydropic degeneration was the term used to describe the pale, vacuolated appearance of the nerve head and retro-laminar tissues during the first few days before the development of frank cavernous degeneration. In these early lesions an accumulation of acid mucopolysaccharide was not observed; hence the term hydropic degeneration. At least some of the vesicular structures that contributed to the appearance of hydropic degeneration were shown by silver staining to be vacuolated axons. Other axonal enlargements were solid rather than vacuolated. These resembled the end bulbs of Cajal 18, 9 or the cytoid bodies that are also known to correspond to enlarged axons. 19 Much of the vacuolated or spongy tissue observed in the areas of hydropic degeneration could not be adequately evaluated by light microscopy; e.g., it was not possible to determine whether the astrocytes participated along with the axons in the early response to the ischemic damage.

The present electron microscopic study was undertaken in an effort to examine more closely the early stages of optic nerve damage leading to cavernous degeneration. We were particularly interested in the following questions:

1. What corresponds to the vacuolar or spongy change that often precedes the cavernous degeneration of the optic nerve?

2. Is the vitreous indeed forced back into the nerve in acute glaucoma? To help answer this question an electron-dense marker, either India ink (colloidal carbon) or Thorotrast (colloidal thorium dioxide), was injected into the vitreous of the eyes of owl monkeys, and the displacement of these tracers was studied at different time intervals after the onset of experimental glaucoma.

3. What is the fate of the glial cells, axons, and myelin sheaths of the degenerating nerve?

Materials and methods

The "Principles of Laboratory Animal Care" as promulgated by the National Society for Medical Research were observed during this investigation of the optic nerves of 12 owl monkeys (Aotus tri-virgatus). Examination of the eyes of these monkeys prior to the induction of glaucoma revealed normal fundi and an intraocular tension measuring between 17 and 19 mm. Hg Schiötz with a 5.5 Gm. weight. The technique used to produce the glaucoma was the same as previously described. 6 After constriction of the pupils with pilocarpine and under deep pentobarbital anesthesia, the eye was entered at the temporal limbus with a 26 gauge needle mounted on a tuberculin syringe. First, 0.2 c.c. of aqueous humor was withdrawn, and the needle was retracted. Then, 0.12 c.c. of a solution consisting of 750 N.F. units of chymotrypsin (H-Chymar, Armour Pharmaceutical Company, Chicago, Ill.) in 0.4 c.c. of diluent was injected into the posterior chamber by directing the needle through the pupil between iris and lens. Following the injection of alpha-chymotrypsin, 0.2 c.c. of colloidal thorium dioxide (Thorotrast, Testagar & Co., Inc., Detroit, Mich.) was injected into the anterior vitreous with a 26 gauge needle inserted at the limbus, perforating the peripheral iris and avoiding the ciliary body and the relatively small lens. In most monkeys the other eye was treated in the same way, but sterilized India ink (colloidal carbon) instead of Thorotrast was injected into the vitreous. For controls we examined two glaucomatous eyes that had no injection of either Thorotrast or carbon and one nonglaucomatous eye that was injected with the respective tracer. Tonometry, ophthalmoscopy, and slit-lamp examinations were done hourly during the first 6 hours after injection and thereafter at least twice a day.

The monkeys were killed 6, 12 (2 monkeys), 24 (2 monkeys), 36, 48 (2 monkeys), 72, 96, and 168 (2 monkeys) hours after the injection of alpha-chymotrypsin. In most of the eyes of these monkeys glaucoma of varying severity developed after several hours and persisted for the duration of our experiments. Fixation of the tissues was done by glutaraldehyde perfusion. For this purpose the animals were well anesthetized with pentobarbital. The pressure in the eyes was released by a cut into the cornea. Thereafter the chest was opened, the right atrium incised, and a cannula inserted into the left ventricle. The descending aorta was clamped while the fixative was flushing the blood. The perfusion with about 2,000 c.c. of 3.6 per cent phosphate-buffered glutaral-
dehydrate, running under a pressure of 120 mm. Hg, lasted for about 15 minutes. The optic disc and nerve were then carefully dissected and put in a 1 per cent phosphate-buffered osmium tetroxide solution. With the aid of a dissecting microscope smaller blocks were cut from the optic nerve in order to obtain longitudinal and transverse views of the nerve anterior and posterior to the lamina cribrosa. These blocks were left for another 30 minutes in the osmium fixative, then dehydrated and embedded in Epon. All blocks were screened by light microscopy of sections 2 μ thick stained with paraphenylenediamine. Selected blocks from each nerve were trimmed and sectioned for electron microscopy. These sections were vacuumed on bare grids, stained with uranyl acetate and lead citrate, and examined with an RCA EMU 3G electron microscope operating at 50 kv.

Results

The clinical observations did not differ from those previously reported in a study of the optic nerves of 18 monkeys with experimental acute glaucoma that were examined by light microscopy. The intraocular pressure generally began to rise several hours after the injection of alpha-chymotrypsin, reaching a maximum pressure that varied from 40 to 80 mm. Hg after about 12 hours. The injection of India ink was well tolerated, but a few eyes injected with Thorotrast developed an inflammatory reaction.

Optic nerves examined 6 hours after the induction of glaucoma showed no changes...
(Figs. 1 and 2). Eyes without glaucoma injected with either Thorotrast or India ink also revealed no abnormalities of the optic nerve. By electron microscopy these normal nerves showed axons containing neurotubules, a few mitochondria, and vesicular elements. In front of, within, and just behind the lamina cribrosa the axons were embedded within a rich feltwork of astrocytic processes filled with glial filaments. Tight junctions were noted between astrocytes. Otherwise the extracellular space consisted of a small cleft between axonal and glial processes. A few cells rich in ribonucleoprotein particles but devoid of filaments were found in the retrolaminar tissue. These cells, identified as oligodendrocytes, were often arranged in rows in the myelinated portion of the optic nerve. Myelination was complete a short distance from the lamina cribrosa. A few axons surrounded by a thin myelin sheath were occasionally seen within the region of the lamina cribrosa.

Twelve hours after induction of glaucoma, and more strikingly at later stages, the optic nerves showed pathological changes that were most severe in the vicinity of the lamina cribrosa. In most nerves, axons appeared to be selectively affected. Two distinct axonal alterations—hydropic axonal degeneration and reactive axonal enlargement—were observed. Hydropic swelling of axons accounted almost exclusively for the vacuolar changes seen by light microscopy (Figs. 3 and 4). The swollen axons were easily identified when surrounded by a myelin sheath, but in front of and just behind the lamina cribrosa their identification was made with less certainty.

Fig. 5. Prelaminar tissue of optic nerve 24 hours after induction of glaucoma with maximum pressure of 71 mm. Hg. Hydropic degeneration, presumably of an axon, is seen. The arrows point to areas where the axolemma is ruptured. A reactive axon (AX) filled with mitochondria and dense bodies is seen at right. Astrocytic processes (AC) filled with glial filaments are numerous. There is no enlargement of extracellular space. (×18,000.)
(Fig. 5). In early stages the distended axons showed a granular disintegration and clumping of neurotubules and vesicles (Fig. 6). Mitochondria, though often swollen, remained otherwise remarkably well preserved. At later stages completely empty vacuoles surrounded by distended sheaths were noted (Fig. 7). Between such myelin-bounded vacuoles, masses of collapsed sheaths were also seen (Fig. 4). The other, more frequently observed axonal change, which in some nerves developed in the absence of hydropic axonal swelling, consisted of an axonal enlargement filled with mitochondria, dense bodies, membranous whorls, and vesicular elements (Figs. 5, 8, and 9). In some of these reactive axonal enlargements, electron-dense granules resembling glycogen were found within the axoplasm between the accumulated organelles (Fig. 10). No correlation could be established between the severity of the glaucoma and either the hydropic or reactive axonal change.

The glial cells in the vicinity of the affected axons were generally well preserved (Fig. 5). In focal areas, however, some nerves showed a few astrocytes with swollen watery cytoplasm. Behind the lamina cribrosa occasional vacuolated cells were found (Fig. 11). The intracytoplasmic vacuoles were bounded by nuclear and/or cytoplasmic membranes, indicating that the vacuoles represented extremely distended perinuclear spaces and/or distended endoplasmic reticulum. Because of the absence of recognizable filaments within the cytoplasmic rim surrounding the vacuoles, the affected cells were considered to represent oligodendrocytes.

No enlargement of extracellular space was seen during the first 48 hours after glaucoma. At later stages, cystic areas developed focally in some of the most severely affected nerves. These spaces were apparently created after the rupture of the membranes of severely distended axons (Fig. 5) and glial cells. Phagocytosis of cellular debris occurred in these areas (Fig. 12). In one instance we could dem-

Fig. 6. Hydropic degeneration of an axon 12 hours after the induction of glaucoma with a maximum pressure of 71 mm. Hg. The axoplasm shows granular disintegration and clumping of neurofilaments. (×11,000.)

Fig. 7. Hydropic degeneration of axons 24 hours after induction of glaucoma with a maximum pressure of 71 mm. Hg. There is complete disintegration of axoplasm, leaving only distended myelin sheaths. (×4,000.)
Fig. 8. Longitudinal section of reactive axonal enlargements, filled with mitochondria, dense bodies, vesicles, and membranous whorls, 24 hours after induction of glaucoma with maximum pressure of 76 mm Hg. (x13,000.)

Fig. 9. Transverse section of reactive axon, filled with mitochondria, dense bodies, and membranous whorls, 24 hours after induction of glaucoma with maximum pressure of 76 mm Hg. (x11,000.)

Fig. 10. Deposition of glycogen granules within axoplasm among mitochondria, 36 hours after induction of glaucoma with maximum pressure of 61 mm Hg. (x20,000.)
Fig. 11. Vacuolated cell, possibly an oligodendrocyte, in retrolaminar tissue, 36 hours after induction of glaucoma with maximum pressure of 61 mm. Hg. (x5,000.)

Fig. 12. Phagocytosis of cellular debris 24 hours after induction of glaucoma with maximum pressure of 71 mm. Hg. (x6,000.)

donstrate the infiltration of mononuclear cells between separated endothelial cells of a vein, suggesting that at least some of the mononuclear phagocytes originated from hematogenous monocytes (Fig. 13). Reactive astrocytes showing an increased number of ribosomes and mitochondria among bundles of densely packed glial filaments were found at the margin of cystic lesions.

Neither Thorotrast nor carbon particles were noted within most of the damaged nerves. The markers were apparently prevented from penetrating into the nerve as long as the internal limiting membrane covering the nerve head remained intact (Figs. 14 and 15). In 3 nerves of 2 monkeys put to death 7 days after the induction of glaucoma, however, the markers were found within extracellular spaces and phagocytes. One of these nerves had progressed to severe cavernous degeneration that also involved the myelinated portion of the nerve (Figs. 16 to 19). The cavernous changes consisted of enormously widened extracellular spaces that were filled with finely granular material, presumably vitreous, containing the respective markers (Figs. 20 and 21). The penetration of vitreous and the respective markers into the damaged nerve after the rupture of the internal limiting membrane could be demonstrated in serial sections (Fig. 17). Once past this membrane, the injected markers spread throughout extracellular spaces, forming massive deposits within the degenerated nerve bundles but without penetrating into the connective tissue septa (Fig. 22). A few phagocytes containing Thorotrast or carbon particles, as well as myelin and degenerated axons, within membrane-bounded compartments inside their cytoplasm were floating within the cavernous spaces (Fig. 22). Phagocytes were also seen penetrating beneath distended myelin sheaths via nodes of Ranvier (Fig. 23). Following this pathway the cells came to lie within myelin-bounded vacuoles or between myelin sheaths and the degenerating axons (Figs. 24 and 25). This was observed at the margin of cavernous spaces within still compact though vacuo-
Fig. 13. Penetration of mononuclear cells between endothelial cells (E) of a large vein, 72 hours after the induction of glaucoma with a maximum pressure of 50 mm Hg. BM = Basement membrane. (x13,000.)

Discussion

Previous light microscopic studies on experimental acute glaucoma demonstrated a variety of changes in the damaged optic nerves. During the first 4 days, the nerves showed vacuolar degeneration and confluent microcysts suggestive of an early cavernous degeneration but without accumulation of acid mucopolysaccharides. After 4 to 7 days, we observed nerves showing cavernous degeneration characterized by enormously distended extracellular spaces filled with acid mucopolysaccharides that stained similarly to vitreous. The studies reported here confirmed these observations by showing that electron-dense markers injected into the vitreous do not penetrate into the nerve until after the dissolution or rupture of the internal limiting membrane secondary to the cystic transformation of the underlying necrotic tissue.

In all the damaged nerves, regardless of whether they show vacuolar, cystic, or cavernous changes, there are axons with reactive alterations consisting of an accumulation of mitochondria, dense bodies, vesicles, and often also membranous whorls. We have chosen the term reactive to emphasize that this type of axoplasmic alteration always occurs in axons close to and at sites of injury; e.g., it characteristically develops in the distal and proximal stumps of severed axons. It is possible, however, that the accumulated axoplasmic organelles...
Fig. 14. Aggregates of Thorotrast particles in vitreous body, 24 hours after induction of glaucoma with maximum pressure of 71 mm. Hg. The intact internal limiting membrane (ILM) prevents the penetration of the particles into the nerve. (×10,000.)

Fig. 15. Cystic transformation of necrotic tissue underlying the internal limiting membrane, 96 hours after the induction of glaucoma with maximum pressure of 64 mm. Hg. (×5,000.) The arrow points to an area that is shown in an adjacent section at higher magnification in the inset. Inset: A markedly thinned internal limiting membrane (arrow) is the only barrier left between the vitreous body and the underlying cystic space. Finely granular material is present within the nerve beneath the internal limiting membrane. This material may represent vitreous that has already penetrated into the nerve. (×22,000.)
do not actively proliferate as a reaction to injury but that they passively aggregate because of an axoplasmic stasis at the site of injury. If this should be proved, we are prepared to change the term reactive to stagnant axoplasmic alteration in order to be more specific. Within hours after injury, axons enlarge and fill—first with mitochondria and vesicles and later also with electron-dense bodies and filaments. In distal segments of transected axons these organelles then undergo degenerative changes. Filaments and vesicles show granular disintegration. Mitochondria, though often remaining remarkably well preserved, eventually change to membranous dense bodies that conglomerate to form membrane-bounded flocular structures. Whorls of membranes similar to those described in this report have been ob-
Fig. 20. Prelaminar tissue of a nerve undergoing cavernous degeneration 7 days after induction of glaucoma with a maximum pressure of 42 mm. Hg. There are very wide extracellular spaces (ES) that are filled with finely granular material, presumably vitreous, containing carbon particles. A macrophage (MP) containing carbon particles within membrane-bounded compartments is seen in the left upper corner. A small reactive axon (AX) and astrocytic processes (AC) are also seen. (x16,000.)

Fig. 21. Cavernous extracellular spaces are filled with aggregates of Thorotrast particles. This picture was obtained from the area marked with an arrow in Fig. 19. Transverse sections of three axons, showing collections of distorted mitochondria within degenerated axoplasm, are seen. (x7,000.)
served in transected optic nerves and in damaged axons in the retina. The accumulation of glycogen granules within the axoplasm is also known to occur in degenerating nerves. The fact that axons are indeed interrupted in acute glaucoma is indicated by the presence of focal areas of ischemic necrosis. It may further be assumed that axonal flow is interrupted in axons that show severe hydropic distension. Since it is also known that reactive axonal enlargements develop at sites of compression or ligation of nerves, one should think about the possibility that less markedly raised intraocular pressure might interfere with axonal flow even before blood flow is sufficiently reduced to cause ischemic axonal damage. However, in our experiments comparison of the severity of the glaucoma and the occurrence of predominantly reactive or hydropic axonal changes provided no correlation.

Glial cells are less vulnerable to ischemia than axons, a fact that has also been demonstrated in retinal ischemia. In focal areas, however, some optic nerves showed necrotic cells that were phagocytosed. At the margin of these foci, astrocytes showed reactive changes consisting of cytoplasmic swelling followed by a proliferation of ribosomes, mitochondria, and glial filaments. These cells did not accumulate glycogen, however, a feature that makes their behavior different from that of protoplasmic astrocytes after injury to the cerebral cortex. In the retrolaminar tissue of the optic nerve there were also a few vacuolated cells showing enormously distended endoplasmic reticulum. Changes of this kind have been described in oligodendrocytes in prolonged edema of the brain, but we have also seen this alteration in areas of poor fixation, though not to the extent illustrated in Fig. 11. Phagocytes were observed at the margin of necrotic tissue after about 2 days. In nerves showing cavernous degeneration, these cells remained relatively scanty, suggesting that the accumulated acid mucopolysaccharides and/or the propagated pressure from the vitreous somehow interfere with the proliferation or infiltration of the phagocytes. Much controversy exists about the origin of these cells. We believe that they are derived both from hematogenous monocytes and from reticuloendothelial cells (microglia) that were stationed in the tissue prior to injury. Radio-
Fig. 23. A mononuclear phagocyte (MP) penetrates (arrow) beneath a myelin sheath via a node of Ranvier in a degenerating nerve 7 days after induction of glaucoma. (×7,000.)

Fig. 24. A mononuclear phagocyte has penetrated beneath a distended myelin sheath after complete hydropic disintegration of the axon in a damaged nerve 7 days after induction of glaucoma. (×10,000.)

Fig. 25. A mononuclear phagocyte has penetrated between a distended myelin sheath and the shrunken degenerated axon (AX) in a damaged nerve 7 days after induction of glaucoma. (×10,000.)
autographic studies support this view. The electron micrograph illustrating the invasion of mononuclear cells through the wall of a vein (Fig. 13) certainly is consistent with the interpretation of a hematogenous origin of some of these cells. Similar invasions by mononuclear cells through the walls of venules are more readily observed in inflammatory diseases of the brain.

The phagocytes engulfed degenerated axons, cellular debris, and the injected markers. The removal of myelin from degenerated axons occasionally proceeded in a rather spectacular fashion. The phagocytes penetrated beneath the sheath via a node of Ranvier. In this fashion mononuclear cells came to lie between axons and their sheath, or—after hydropic disintegration of the axoplasm—the cell was lying within the empty space surrounded by a myelin sheath. The invasion of mononuclear cells into hollow tubes of myelin sheaths after axonal disintegration has been observed by other investigators. In demyelinating diseases this type of myelin removal, i.e., the stripping of intact myelin fragments by invading mononuclear cells, has been recognized as a nonspecific mechanism of demyelination that always occurs after injury to myelin sheaths or possibly also after degeneration of the myelin supporting oligodendrocytes.

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