Pathology of the optic nerve in experimental acute glaucoma

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Damage to the optic nerve resulting from severe acute glaucoma was studied experimentally in the owl monkey (Aotus trivirgatus). Soon after the onset of glaucoma a widespread marked reduction in blood flow was evident in the optic nerve as well as in the ocular tissues. During the first 4 days there was progressive hydropic degeneration of the nerve head beginning in the vicinity of the lamina cribosa, but extending back into the nerve, mainly in the axial zone. Within 4 to 7 days extensive areas of cavernous degeneration were observed posterior to the lamina, extending back 3 to 6 mm. The nerve head appeared congested and edematous during the first week. Atrophy and early cupping became apparent after 1 week and definite cupping after 2 to 3 weeks. While the areas of cavernous degeneration were rich in acid mucopolysaccharide sensitive to hyaluronidase and typically free of microglial and astrocytic reaction during the first week, after 2 to 4 weeks the cellular response produced a picture resembling infarction. It was concluded that cavernous degeneration of the optic nerve is a peculiar form of ischemic necrosis developing as a consequence of severe acute glaucoma and that the hyaluronic acid, probably forced into the optic nerve from the vitreous, may be one of several factors responsible for the unusual histopathologic picture that characterizes this form of infarction.

Hamasaki and Ellerman, in their experimental study of the effect of alpha chymotrypsin on the retina, observed that in some animals the enzyme produced a dislocation of the lens and a rise in intraocular pressure without gross alteration in the electroretinogram. Subsequently Kalvin, Hamasaki, and Gass showed that in the owl monkey (Aotus trivirgatus) an open-angle glaucoma could be produced with regularity by injecting into the posterior chamber 75 to 375 units of alpha chymotrypsin. The treated eyes became glaucomatous for the duration of their experiments (up to several weeks). The authors claimed that cupping of the optic disk was observed ophthalmoscopically after 7 days. Histopathologic studies also revealed cupping and, in addition, cavernous degeneration of the optic nerve. That the optic nerve changes represented a complication of elevated intraocular pressure rather than a specific effect of the alpha chymotrypsin was demonstrated by other experiments in
which the enzyme was injected into the posterior vitreous. In these experiments severe degeneration of the photoreceptors was observed, but the eyes did not become glaucomatous, and the optic nerves exhibited no evidence of cupping or of cavernous degeneration. Furthermore, changes in the optic nerve similar to those found in eyes made glaucomatous with alpha chymotrypsin were observed in eyes that had been made glaucomatous by other techniques not involving the use of alpha chymotrypsin. Evidence was obtained in these experiments that the optic nerve changes were the consequence of a marked reduction in blood flow into the eye and distal part of the optic nerve. The resultant areas of Schnabel's cavernous degeneration were interpreted as infarcts of the nerve.

Schnabel's cavernous degeneration, as seen in the human optic nerve, is characterized histopathologically by the presence of an acid mucopolysaccharide that is sensitive to hyaluronidase within the cavernous spaces, and clinically by its relationship to glaucoma. Infarcts of the optic nerve observed in nonglaucomatous eyes do not exhibit an accumulation of hyaluronic acid in the necrotic nerve head. In an effort to study the sequence of changes leading to cavernous degeneration of the optic nerve and with the hope of determining the source of the hyaluronic acid contained in the cavernous spaces in Schnabel's optic atrophy, another series of experiments was undertaken. It is the purpose of this paper to record and illustrate the gross and light microscopic changes that we have observed to date.

Materials and methods

The basic technique used for the production of acute glaucoma in the owl monkey (Aotus trivirgatus) was that of Hamasaki and co-workers. The monkeys were examined carefully bio-microscopically, gonioscopically, ophthalmoscopically (direct and indirect), and tonometrically the day before the experiments were begun, and a fundus photograph of each eye was obtained. Mydriacyl (1 per cent) was used to dilate the pupils for these examinations.

The next day pilocarpine (1/4 per cent) was used to constrict both pupils. Within 15 to 30 minutes the pupils were narrowed to pinpoints. Nembutal (0.2 c.c.) was given intraperitoneally, and the experiments were begun 30 minutes later. A fixation forceps was used to grasp the limbus temporally. First 0.2 c.c. aqueous was withdrawn from the anterior chamber of the right eye with a 26-gauge needle and a tuberculin syringe. The needle was introduced in the peripheral 2 mm. of cornea temporally. After withdrawal of aqueous the needle was reintroduced into the same tract. It was passed through the constricted pupil, between the nasal iris leaf and the lens, and into the posterior chamber, into which 225 units of alpha chymotrypsin* (0.12 c.c.) was injected. The same procedure was used on the left eye except that diluent or heat-inactivated enzyme was injected for control studies. Following this procedure the anterior chamber usually was restored to its initial depth.

During the first day after the experiments were begun each animal was examined at least hourly for 6 hours and often the examinations were much more frequent than this. Afterwards the monkeys were examined at least twice daily and the pressures recorded in each eye. These daily examinations also included ophthalmoscopy (direct and indirect), slit-lamp examination, and gonioscopy when indicated.

Initially 2 monkeys were painlessly put to death at about 1/4, 1, 2, 4, 7, 14, and 21 days following the onset of glaucoma. Subsequently additional experiments were performed to provide more observations 3 to 4 days after onset of glaucoma, and one monkey was killed after 28 days. After the monkeys had been anesthetized with 0.2 c.c. of Nembutal intraperitoneally, a thoracotomy was performed to expose the heart, and the animals were killed by the rapid intracardiac injection of 10 c.c. of India ink (Pelikan). Death usually occurred within a minute or two. The eyes were enucleated with 7 to 10 mm. of optic nerve attached and fixed in 10 per cent formalin.

Originally, it was planned to put ferritin into the posterior chamber along with the Zolyse and to fix the eyes in glutaraldehyde to permit both light and electron microscopic studies. The ferritin was to serve as a tracer, mainly for electron microscopic studies. Those eyes receiving ferritin, however, developed a severe endophthalmitis soon after the experiments were begun even though cadmium-free ferritin was used. The use of ferritin

*The "Principles of Laboratory Animal Care" as promulgated by the National Society for Medical Research were observed during this investigation.

*Zolyse, manufactured by Alcon Laboratories, Inc., Fort Worth, Texas, was used for all our experiments.
was, therefore, discontinued in these experiments. None of the observations to be reported were made on eyes that had received ferritin.

The eyes were opened in the horizontal plane after several days of fixation. Paraffin sections were cut anteroposteriorly in the horizontal plane at 8 to 14 μ and the following stains were used: hematoxylin and eosin, Masson's trichrome, the Verhoeff-van Gieson for connective tissue, Bodian's method for axis cylinders, Weil's and Woelcke's methods for myelin, Luxol fast blue, alcian blue with and without hyaluronidase, colloidal iron method for acid mucopolysaccharides with and without hyaluronidase, periodic acid-Schiff reaction, and the Prussian blue reaction for iron. In a few selected cases a combination of stains for acid mucopolysaccharide and for myelin was used. The thicker sections (10 to 14 μ) gave more satisfactory staining reactions for axons and myelin.

The calottes were used for the study of retinal vessels by the method of Kuwabara and Cogan after digestion in trypsin.

Observations

Clinical. In the present report we shall be concerned mainly with the effect of the experimental glaucoma on the optic nerve. It is necessary, however, to record briefly the sequence of events observed clinically after the injection of alpha chymotrypsin or control materials into the posterior chamber. In the control eyes that received diluent or heat-inactivated enzyme, the pupils remained markedly constricted for at least 12 hours and there was usually no significant evidence of ocular inflammation or secondary glaucoma. Exceptions were the eyes that received ferritin and two control eyes that sustained an injury to the lens and developed a pupillary block with severe secondary glaucoma. Almost all eyes that received alpha chymotrypsin exhibited a distinctive pattern of response. Soon after the enzyme was introduced (within 15 to 60 minutes) the pupil began to dilate, and long before any evidence of pupillary dilatation was observed in the control eye, the pupil reached maximum dilatation. Shortly before or, occasionally, after this dilatation of the pupil became maximal, the intraocular pressure began to rise rapidly. Within 3 to 4 hours pressures were in the range of 32 to 37 mm. Hg (Schiotz), and by 6 to 8 hours they generally exceeded 50 mm. Hg. It was quite apparent that, whenever the intraocular pressure exceeded 50 mm. Hg, the cornea became edematous and this interfered with ophthalmoscopy.

In about one half of the glaucomatous eyes changes in the ocular media prevented adequate ophthalmoscopic evaluation. In the remaining cases, the ophthalmoscopically visible changes were variable and appeared to depend on the height of the intraocular pressure and the time required to reach an intraocular pressure of 70 mm. Hg. When a rise in pressure to 70 mm. Hg occurred within 8 hours, pallor of the disk was the initial change observed. In these cases retinal edema, either diffuse or focal, was seen within 12 to 18 hours. In most of the cases, however, congestion of the disk and retinal vessels appeared in 12 to 15 hours. This congestion persisted for 4 to 5 days and then pallor of the disk was observed. Glaucomatous cupping was observed only after 9 to 10 days. In those eyes that developed thrombosis of the

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*Monkey 7 was put to death 3 weeks after the onset of glaucoma, but the intraocular pressure had returned to normal levels after 2 weeks of severe glaucoma.
central retinal vein (to be discussed elsewhere) retinal hemorrhages were seen initially at the equatorial region after 12 hours of acute glaucoma. The retinal hemorrhage became extensive at 24 hours. Table I summarizes pertinent data concerning the duration of glaucoma, maximum intraocular pressure, and intraocular pressure at the time the experiments were terminated.

Pathologic. The monkeys were put to death by the intracardiac injection of India ink in an effort to compare the blood flow into the two eyes at the time the experiments were terminated. When the formalin-fixed pairs of eyes were opened there was seldom any difficulty in determining which were the glaucomatous eyes because they almost always showed much less filling of the retinal vessels with India ink than the normotensive fellow eyes (Fig. 1). In a few cases retinal hemorrhages were present. Retinal vascular complications of this experimental acute glaucoma will be considered elsewhere. Macroscopic examination of the optic disks did not usually reveal any distinctive differences between the two eyes during the first week. Pallor and/or cupping were noted in some cases 2 to 3 weeks after the onset of glaucoma.

Microscopically it was also readily apparent in the majority of cases that the capillaries as well as the larger vessels contained less India ink in the glaucomatous eyes (Figs. 2 to 4). Not only was this true of the intraocular capillaries, but frequently the capillaries of the optic nerve several millimeters posterior to the lamina cribrosa also revealed a conspicuous difference in their content of India ink (Fig. 5).

Twelve hours after the onset of glaucoma (monkeys 19 and 20), minimal hydropic degeneration was observed in the optic nerve fiber bundles immediately posterior to the lamina cribrosa. In monkey 19 there was also a small focus of hydropic degeneration adjacent to the central retinal vessels, on their temporal side, about 1 mm. posterior to the lamina. In monkey 20 there was very mild papilledema, and a few markedly swollen nerve fibers resembling retinal cytoid bodies were identified in the retrolaminar area of hydropic degeneration.

Twenty-four hours after the onset of glaucoma (monkeys 17 and 18), papilledema (Fig. 6) was observed in both cases. A fibrinous thrombus was found in both central retinal veins, but in only one of the two eyes (monkey 17) were there any retinal hemorrhages and in this case microhemorrhages were also present in the edematous nerve head. Moderate hydropic degeneration was observed within and immediately behind the lamina cribrosa. In monkey 18 axonal swellings were noted in the swollen nerve head (Fig. 7) and in the retrolaminar area (Fig. 8).

After 2 days, monkey 32 had mild papilledema with a focus of early cavernous degeneration in the temporal portion of the nerve head (Fig. 9). Moderate hydropic degeneration...
Fig. 2. A. The central retinal vessels of the control eye of monkey 27 are well filled with India ink. (Hematoxylin and eosin, ×80. AFIP Neg. 65-13059.) B. The treated eye, glaucomatous for 48 hours, has central retinal vessels that are poorly filled with India ink. (Hematoxylin and eosin, ×80; reduced ½. AFIP Neg. 65-13060.)

Fig. 3. A. The retinal vessels of the control eye of monkey 20 are well filled with India ink. B. The retinal vessels in the treated eye, glaucomatous for 12 hours, contain no India ink. (Aniline blue, ×115; reduced ½. AFIP Neg. 66-183.)

degeneration of nerve fiber bundles was present in the retrolaminar area (Fig. 10), and axonal swellings were found in this area nasally. Similar changes, including the presence of cytoid bodies, were observed in monkey 27. In this monkey there was also a focal area of hydropic degeneration along the temporal side of the central vessels farther back in the nerve.

Both eyes (monkeys 34 and 35) studied 3 days after the onset of glaucoma revealed papilledema with severe hydropic degeneration within and immediately behind the

Fig. 4. Flat preparation of whole retinal vessels after digestion of retina by trypsin (same monkey as in Fig. 3). (Hematoxylin and eosin, ×80; reduced ½.) A. Most of the retinal capillaries of the normal control eye are well filled with India ink. (AFIP Neg. 66-4083.) B. No India ink is present in the retinal vessels of the glaucomatous eye. (AFIP Neg. 66-4081.)
Fig. 5. These fields of the optic nerves from the same monkey as Figs. 3 and 4 were photographed several millimeters posterior to the lamina cribrosa. (Aniline blue, ×115; reduced ½. AFIP Neg. 66-184.) A, Many of the capillaries of the control eye are filled with India ink. B, Only a rare capillary of the glaucomatous eye is filled with India ink.

lamina cribrosa (Fig. 11). In monkey 34 the hydropic degeneration extended back into the anterior part of the nerve for a considerable distance, especially in the axial portion. Anteriorly a few swollen axons were observed (Fig. 12), while posteriorly there were numerous large vesicular structures (Fig. 13, A and B). Bodian stains for axis cylinders and stains for myelin revealed these vesicular structures to be the extremely swollen ends of interrupted nerve fibers (Fig. 13, C). No accumulation of acid mucopolysaccharide was evident at this stage. In monkey 35 there was a thrombus in the central retinal vein, and the retina showed evidence of massive hemorrhagic infarction. This eye also had hemorrhages in the nerve head. The hydropic degeneration extended back from the laminary area a short distance, and a small accumulation of acid mucopolysaccharide sensitive to hyaluronidase was present. A few cytoid bodies were seen farther back in the central part of the nerve.

All three eyes (monkeys 21, 36, and 37) examined 4 days after the onset of glaucoma revealed papilledema (Fig. 14). In
Fig. 9. Early cavernous degeneration of optic nerve head 2 days after onset of glaucoma in monkey 32. (Hematoxylin and eosin. ×305; reduced ½, AFIP Neg. 66-4099.)

Fig. 10. Severe hydropic degeneration of nerve fibers in the laminary and retrolaminar area of the nerve head of same eye shown in Fig. 9. Note the very poor staining of axons in this area. (Bodian stain. ×200; reduced ½. AFIP Neg. 66-4098.)

Fig. 11. Severe hydropic degeneration is present throughout the nerve head and in the anterior part of the retrolaminar optic nerve, axially. Monkey 34, 3 days after onset of glaucoma. (Hematoxylin and eosin. ×100; reduced ½. AFIP Neg. 66-483.)

Fig. 12. Widespread destruction of nerve fibers is evident, and several focal areas of severe swelling can be seen (arrows). Same nerve shown in Fig. 11. (Bodian stain. ×380; reduced ½. AFIP Neg. 66-3953.)

2 (monkeys 36 and 37) there was severe hydropic degeneration involving the nerve fiber bundles within and on both sides of the lamina cribrosa (Figs. 14 and 15). In monkey 36 there were cytid bodies in the nerve head and in the anterior part of the nerve proper, and a few hemorrhages were present behind the lamina. Hydropic degeneration extended back farther into the nerve axially. In monkey 37 the changes, including a focus of hemorrhage behind the lamina, were similar to those observed in monkey 36 except that cytid bodies were not observed. In monkey 21 the picture was dramatically different, for in this case there was massive cavernous degeneration beginning just behind the lamina and extending back about 6 mm. (Figs. 16 to 20). The process thickened the nerve, and only a few nerve fiber bundles along the temporal edge of the nerve seemed to be spared. The cavernous spaces were filled with an acid mucopolysaccharide that was sensitive to hyaluronidase (Fig. 17), and the process in general appeared to be identical to Schnabel's cavernous optic atrophy observed in certain glaucomatous human eyes. In one area intense engorge-
Fig. 13. A, Many vesicular structures are present in the parenchyma of the same optic nerve shown in Figs. 11 and 12. (Hematoxylin and eosin. ×40. AFIP Neg. 66-3948.) B and C, The Bodian stain reveals these vesicular lesions (arrows) to be markedly swollen degenerating nerve fibers. (×380. AFIP Negs. 66-3951 and 66-3950, respectively.)

Fig. 14. Papilledema in monkey 37, 4 days after onset of glaucoma. There is severe hydropic degeneration of the nerve fiber bundles in the nerve head, in the laminary area, and in the optic nerve, especially axially. (Hematoxylin and eosin. ×80; reduced ⅔. AFIP Neg. 66-477.)

Fig. 15. Severe hydropic degeneration is evident in the nerve head, in the lamina cribrosa, and in the retrolaminary area of monkey 36, 4 days after onset of glaucoma. (Hematoxylin and eosin. ×130; reduced ⅔. AFIP Neg. 66-479.)
Fig. 16. A, Extensive cavernous degeneration of the optic nerve in monkey 21, 4 days after onset of glaucoma. (Hematoxylin and eosin. ×14; reduced ½. AFIP Neg. 65-13142.) B, The colloidal iron stain for acid mucopolysaccharides is strongly positive (dark areas in the nerve). (×14; reduced ½. AFIP Neg. 65-13143.)

Fig. 17. Cross section of the optic nerve shown in Fig. 16 taken about 6 mm. posterior to lamina cribrosa. A, With the routine hematoxylin-eosin stain, either the cavernous spaces appear empty or they contain only a faintly stained serous material. (×35; reduced ½. AFIP Neg. 65-13150.) B, The colloidal iron stain for acid mucopolysaccharides is strongly positive. (×35; reduced ½. AFIP Neg. 65-13153.) C, After treatment with hyaluronidase, the colloidal iron reaction is negative. (×35; reduced ½. AFIP Neg. 65-13151.)

Fig. 18. A, Although there is massive disintegration of the optic nerve posterior to the lamina cribrosa, the nerve head is remarkably well preserved. Same nerve as shown in Figs. 16 and 17. (Periodic acid–Schiff–hematoxylin. ×50; reduced ½. AFIP Neg. 66-280.) B, The Bodian stain also reveals remarkably well-preserved neurites in the nerve head, but complete disappearance of nerve fibers posterior to the lamina. (×130; reduced ½. AFIP Neg. 66-4084.)
Fig. 19. A, On the left, many nerves with intact myelin sheaths are still present, but in the areas of advanced cavernous degeneration (on right) myelin appears to have largely vanished. Same optic nerve as shown in Figs. 16 to 18. (Myelin stain. ×50; reduced ¼. AFIP Neg. 66-4085.) B, Partial demyelination and vacuolar degeneration of nerve fibers (arrows) is shown in this transitional zone along the edge of an area of severe cavernous degeneration. (Myelin stain. ×380; reduced ¼. AFIP Neg. 66-193.)

Fig. 20. A, Bodian stain reveals a few remaining axons along the edge of a large area of cavernous degeneration. Same optic nerve shown in Figs. 16 to 19. (×305; reduced ¼. AFIP Neg. 66-4086.) B, Huge globoid bodies (arrows) indicate sites where axons are interrupted. (Bodian stain. ×450; reduced ¼. AFIP Neg. 66-188.)

ment of capillaries and small hemorrhages were observed. In this area there were also focal collections of polymorphonuclear leukocytes in the nerve and in the leptomeninges. It was very strikingly apparent that, even though the anterior end of the optic nerve was largely devastated, the nerve head was remarkably well preserved (Fig. 18). The Bodian stain revealed intact axons in the retinal nerve fiber layer and in the nerve head, but posterior to the lamina cribrosa the nerve fibers were largely destroyed. Further back in the nerve varying stages of dissolution of the nerve fibers could be observed (Figs. 19 and 20). Strangely, little evidence of debris from destroyed nerve fibers and no cellular inflammatory response to the necrosis of nerve tissue was apparent.

In the two eyes studied 1 week after the onset of glaucoma (monkeys 22 and 30), the nerve heads appeared atrophic, and
there was slight posterior bowing of the lamina cribrosa. Extensive cavernous degeneration, beginning at the lamina and extending back 3 mm. (Fig. 21), was seen in monkey 22. Acid mucopoly saccharide sensitive to hyaluronidase was present (Fig. 22). Focal hemorrhages and early gliosis were also noted. In the case of monkey 30, the eye had been glaucomatous for 7 days, with intraocular pressure up to 82 mm. Hg, but the tension fell to normal the day the animal was killed. In this eye the vessels of the retina and optic nerve were unusually dilated and well filled with India ink (Fig. 23), containing even more than the normal control. There was no cavernous degeneration, but special stains revealed extensive axonal degeneration and demyelination (Figs. 24 and 25).

Fig. 21. One week after onset of glaucoma this eye from monkey 22 reveals atrophy of the disk with very early cupping. Posteriorly there is advanced cavernous degeneration. (Hematoxylin and eosin. x13; reduced ½. AFIP Neg. 66-242.)

Fig. 22. Same specimen as shown in Fig. 21. A, The area of cavernous degeneration gives an intensely positive staining reaction for acid mucopolysaccharide. B, This section, after treatment with hyaluronidase, gives a negative reaction for acid mucopolysaccharide. (Colloidal iron stain. >8; reduced ½. AFIP Neg. 66-223.)

Fig. 23. This eye from monkey 30 had been glaucomatous for 1 week, but the intraocular pressure had returned to normal the day the animal was put to death. The blood vessels are unusually well filled with India ink. There is mild atrophy and early cupping of the optic disk. (Nuclear fast red. x40; reduced ¼. AFIP Neg. 66-4093.)

Fig. 24. Same eye as shown in Fig. 23. Atrophy of the disk, early cupping, and mild demyelination of the anterior part of the nerve, particularly in the axial region, are evident. (Myelin stain. >50; reduced ¼. AFIP Neg. 66-4094.)

Fig. 25. Same eye as shown in Figs. 23 and 24. The Bodian stain reveals marked axonal degeneration in the axial portion of the nerve. (>380; reduced ½. AFIP Neg. 66-4068.)
extending back several millimeters, and there was mild diffuse gliosis.

Of the two monkeys studied 2 weeks after the onset of glaucoma, one (monkey 11) revealed definite cupping with atrophy of the nerve head (Fig. 26). In this case extensive necrosis of the optic nerve presented a mixture of features of cavernous degeneration (Fig. 26) and infarction (Fig. 27). There was considerable acid mucopolysaccharide sensitive to hyaluronidase, but there were also numerous microglia, which typically are not observed in Schnabel's cavernous degeneration. These macrophages contained some phagocytosed myelin and an acid mucopolysaccharide that was resistant to hyaluronidase. Monkey 12 had had intraocular pressures up to 82 mm. Hg, but the pressure was down to 32 mm. Hg when the experiment was terminated. There was no cupping, but the juxtapapillary nerve fiber layer of the retina revealed gliosis. An area of intense microglial reaction was present just posterior to the lamina cribrosa. Special stains revealed widespread neuronal degeneration extending back more than 3 mm., yet the posterior part of the nerve, as seen with ordinary stains, appeared fairly normal.

One eye (monkey 2) studied 3 weeks after the onset of glaucoma revealed deep cupping and massive necrosis extending back about 3 mm. posterior to the lamina. A small amount of acid mucopolysaccharide sensitive to hyaluronidase was still present, but there was marked gliosis and numerous microglia were observed (Fig. 28). The other monkey that was sacrificed after 3 weeks (monkey 7) had had glaucoma for 2 weeks with pressures up to 71 mm. Hg, but during the last week of the experiment pressures varied between 15 and 23 mm. Hg. Microscopic examination of the eye revealed no cupping, but the disk was atrophic (Fig. 29, A). There was an extensive area of necrosis of the optic nerve extending back about 2.5 mm. posterior to the lamina cribrosa. This area gave a faintly positive staining reaction for acid mucopolysaccharide (sensitive to hyaluronic acid) and a negative reaction for acid mucopolysaccharide resistant to hyaluronidase (Fig. 29, B).

**Fig. 26.** Glaucomatous cupping after 2 weeks in monkey 11. Cavernous degeneration is present temporally. (Hematoxylin and eosin, ×80; reduced ¼. AFIP Neg. 66-225.)

**Fig. 27.** A, Same optic nerve as shown in Fig. 26 but several millimeters posteriorly where the cavernous degeneration involves the full thickness of the nerve. The cellular infiltrate in the area indicated by arrows is shown at higher magnification in B. (Hematoxylin and eosin, ×50; reduced ¼. AFIP Neg. 66-224.) B, The microglial reaction here is characteristic of infarction. (Hematoxylin and eosin, ×305; reduced ¼. AFIP Neg. 66-279.)
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Fig. 28. A, A large area of necrosis several millimeters posterior to the lamina cribrosa in monkey 2, put to death 3 weeks after onset of glaucoma. The area between arrows is shown at greater magnification in B. (Hematoxylin and eosin, ×80; reduced 1/3, AFIP Neg. 66-231.) B, Proliferation of histiocytes and astrocytes is marked. (Hematoxylin and eosin, ×305; reduced 1/3, AFIP Neg. 66-230.)

ronidase). There were foci showing considerable gliosis, and a moderate infiltrate of microglia was present (Fig. 29, B). These macrophages contained phagocytized myelin and an acid mucopolysaccharide that was resistant to hyaluronidase.

The one eye studied 4 weeks after the onset of glaucoma (monkey 33) revealed deep cupping of the disk and severe cavernous degeneration extending back 5 mm. behind the lamina cribrosa (Fig. 30, A). Large amounts of hyaluronic acid were present in the cavernous spaces, but there was also marked gliosis anteriorly (Fig. 30, B). Microglia, however, were few. The retinal architecture was remarkably well preserved (Fig. 31, A), and Bodian stains revealed a well-preserved nerve fiber layer in the juxtapapillary retina (Fig. 31, B).

Fig. 29. A, Optic atrophy in monkey 7, put to death 3 weeks after onset of glaucoma (but intraocular pressures had been in normal range for 1 week). The large pale area posteriorly is the result of marked demyelination. (Verhoeff-van Gieson stain, ×13; reduced 1/3, AFIP Neg. 66-237.) B, A portion of the pale area of demyelination shown in A. There is cavernous degeneration, but a light infiltrate of microglia is present. (Hematoxylin and eosin, ×305; reduced 1/3, AFIP Neg. 66-232.)

Discussion

It is of interest that the initial optic nerve changes that we observed in our experiments with acute glaucoma were in the vicinity of the lamina cribrosa, and that papilledema preceded optic atrophy and glaucomatous cupping. While the occurrence of papilledema in acute glaucoma is not widely recognized, it has been well documented since the early studies of Schnabel and Elschnig. They empha-
sized that the earliest changes in the optic nerve are to be found in front of, within, and just behind the lamina cribrosa, and that during this early phase there is no posterior bowing of the lamina cribrosa. Their excellent photomicrographs reveal papilledema in these early cases, and Elschnig described the process as a neuritis-like swelling of the disk tissues and adjacent retina. It has long been postulated that obliteration of the capillary bed in the vicinity of the lamina cribrosa is the cause of these early changes, that the latter progress to cupping, and that cavernous degeneration follows excavation of the disk.

The observations we have made in our experiments are not consistent with this sequence of events. We observed extensive cavernous degeneration extending back as far as 6 mm. posterior to the lamina cribrosa before there was even unequivocal bowing of the lamina. The severity of the optic nerve damage and the rapidity with which the nerve disintegrated were surprising. Most surprising, however, were the facts that the vascular effects of the acute glaucoma were so profound so far posterior to the eye, and that axons could be so well preserved in the nerve head (Fig. 18) and retina (Fig. 31), even though there was severe destruction of the nerve posterior to the lamina cribrosa.

Clinicians interested in the mechanism by which glaucoma produces visual damage have suggested that a marked rise in intraocular pressure tends to affect mainly the blood supply to the nerve head. Kalvin and co-workers provided experimental observations that supported the belief that acute glaucoma does lead to very marked venous stasis and ischemia in the eye. Our observations confirmed theirs, but additionally, they suggest that the hemodynamic disturbance produced is much more widespread and that the optic nerve posterior to the lamina cribrosa may be even more sensitive to ischemia than the retina and nerve head.

What is the mechanism by which an elevation of intraocular pressure affects the
extraocular circulation in the optic nerve? Several explanations may be postulated: (1) The vasculature in the optic nerve may be affected by a reflex vasospasm. (2) If the optic nerve derives considerable blood supply from the central retinal artery in the owl monkey, as has been shown to be the case in man but not in rhesus monkey, this would be affected by stagnant flow in the central retinal artery resulting from the sudden rise in intraocular pressure. (3) The intraocular pressure may be transmitted through the lamina cribrosa to affect the optic nerve, which, with its tight investment by pia mater, has a limited capacity to swell without production of a marked rise in tissue pressure. (4) Acute hydric degeneration of the optic nerve bundles may produce such a rise in tissue pressure that the capillaries in the pial septa are compressed and obliterated. (5) There may be a combination of the foregoing factors.

With conventional routine histologic staining, one observes first a hydric degeneration in the vicinity of the lamina cribrosa, involving both the prelaminary and the retrolaminar tissues. Often it is possible to identify individual axons that are severely damaged, for they become markedly swollen and exhibit an increased affinity for eosin, fuchsin, and Schiff's reagent after treatment with periodic acid. A cluster of such injured axons resembles a cotton-wool spot in the retinal nerve fiber layer.

Associated with this early hydric degeneration of the nerve bundles in the vicinity of the lamina cribrosa, often a generalized swelling of the disk is seen, with or without minute extravasations of erythrocytes. Fibrinous thrombi may develop in the central retinal vein, leading to hemorrhagic infarction of the retina. Foci of acute hydric degeneration may also be seen farther back in the nerve, particularly adjacent to the central retinal vessels. The earliest changes observed posterior to the lamina were typically in the axial part of the nerve. While the routine hematoxylin-eosin stain always revealed definite pathologic changes, the severity of the neuronal damage could be demonstrated much better with the aid of good Bodian preparations and stains for myelin. This was especially true in those cases in which the intraocular pressure had returned to normal before the animals were sacrificed.

Is Schnabel's cavernous degeneration a special type of infarct resulting from acute ischemia? The evidence would seem to indicate that this is the case. A typical infarct of the optic nerve occurring in the absence of elevated intraocular pressure does appear superficially similar to Schnabel's cavernous degeneration. The most striking differences are the absence of acid mucopolysaccharide in the infarct and the absence of microglia in cavernous degeneration. Whether cavernous degeneration of the optic nerve is identical with the edematous necrosis of the cerebral white matter as described by Peters remains to be determined.

In several of our cases the large destructive lesions had features in common with infarction and cavernous degeneration. These lesions were observed in cases of longer duration. It would appear that acute ischemic infarction occurring in the presence of a marked rise in intraocular pressure is accompanied by the passage of hyaluronic acid from the vitreous through the ischemic tissues of the nerve head into the areas of infarction. Perhaps the hyaluronic acid has an inhibitory effect on the mobilization of microglia and on the proliferation of neuroglia. In two cases (monkeys 12 and 7) the experiments were terminated after 14 and 21 days, respectively, at a time when the intraocular pressures had fallen markedly (from 82 to 32 mm. Hg in monkey number 12, and from 71 to 14 mm. Hg in monkey number 7); in both cases the picture was much more suggestive of infarction than of Schnabel's cavernous degeneration. From our observations it would seem that typical Schnabel's cavernous degeneration is the result of recent ischemic infarction coupled with very
severe elevation in intraocular pressure. The lowering of the intraocular pressure for just a day or two may change the microscopic picture to one resembling infarction (monkeys 2 and 7) or simple optic atrophy (monkeys 12 and 30).

Our observations did not confirm those of Kalvin and associates\textsuperscript{2, 3} suggesting that the temporal side of the nerve is more susceptible to ischemia than the nasal side. Actually, in some of the most extensively damaged nerves, the least damaged tissue was observed temporally. The most striking suggestion of a variation in susceptibility of tissue that we observed was in monkey 33, killed 4 weeks after the onset of glaucoma. While the optic nerve posterior to the lamina was markedly necrotic, the nerve head and juxtapapillary nerve fiber layer of the retina contained remarkably well-preserved axons (Fig. 31, B). Why the optic nerve proper should be more susceptible to acute ischemia than the nerve head and the retina, and why in some cases there is much more evidence of retinal ischemia than in others, are questions that we cannot answer. If the myelinated nerve fibers are more easily damaged than the nonmyelinated, one of these questions would be answered.

REFERENCES

5. Zimmerman, L. E.: In Discussion of Harrington,\textsuperscript{*} pp. 303-308 and color plate (Fig. 80).

Discussion

\textbf{Dr. Henkind.} You have found hyaluronic acid in cavernous atrophy. Is there any hyaluronic acid normally present in the optic nerve, or is it forced back from the vitreous?

\textbf{Dr. Zimmerman.} We assume that the hyaluronic acid in the optic nerve is the result of vitreous being forced back into the nerve. This is an assumption based on two main facts. One of these is that I have shown this lesion to a number of people who have had rather broad experience with various pathologic processes in the brain and spinal cord and they have all told me this is a lesion they have never seen anywhere else. Secondly, I think this is unique to glaucoma and that in ordinary infarcts you do not see it.

\textbf{From the audience.} What is the frequency of this finding? How often do you see this in galaucoma?

\textbf{Dr. Zimmerman.} Since becoming aware of the fact that this can develop so acutely in these ex-
experimental monkeys, we have been paying attention to the situation where we see it in human eyes. Usually the story is that, even though there may have been a history of chronic glaucoma, there is also a history of recent acute pressure rise leading to the enucleation.

From the audience. Have you done any work with causing a gradual rise in pressure?

Dr. Zimmerman. No, we have not.

Dr. Becker. I wonder if it wouldn't be relatively easy to cannulate the optic nerve in these animals and measure the pressure in the nerve. Certainly if it is swollen with vitreous it should be possible to do this and then, in turn, see how this compares with the pressure in the eye.

Dr. Hamasaki. We are doing some experiments in which we put a needle in the optic nerve of monkeys and try to raise the tissue pressure there. In two of the monkeys, when we raised the pressure in the optic nerve, the intraocular pressure also rose.

Dr. Becker. I was asking the question in the other way—what happens in the monkey nerve with high pressure in the globe?

Dr. Hamasaki. We have not done that.