Studies of intraocular pressure

II. The histopathology of experimentally increased intraocular pressure in the rabbit

Carl Kupfer

The aqueous humor outflow channels of the rabbit eye were obstructed by placement of polyethylene tubing in the angle of the anterior chamber. Within 24 hours the intraocular pressure was elevated and remained so for 3 months after which time the animals were killed. Histologic examination verified the selective loss of ganglion cells, loss of myelin from optic nerve fibers, and deep excavation of the optic nerve. It is concluded that experimentally increased intraocular pressure can cause retinal and optic nerve changes in the rabbit eye which duplicate the pathologic changes of the retina associated with elevated intraocular pressure in man.

The histopathologic changes in the retina and optic nerve secondary to elevation of intraocular pressure in man have been well documented. The effect of experimentally increased intraocular pressure upon the retina and optic nerve in the rabbit has received little attention despite the fact that this animal is used in most investigations of the dynamics of aqueous humor. The aqueous humor leaves the rabbit eye by bulk flow through outflow channels in the angle of the anterior chamber, and obstruction of the outflow channels causes elevation of intraocular pressure. The purpose of this paper is to demonstrate that blockage of the outflow channels in the rabbit eye produces a sustained elevation of intraocular pressure, and, subsequently, a loss of retinal ganglion cells, demyelination of their axons, and excavation of the optic nerve.

Materials and methods

Ten albino rabbits weighing 2.5 to 3.5 kilograms were anesthetized with intravenously administered pentobarbital (40 mg. per kilogram). A 5 mm. groove was made in clear cornea 3 mm. from the limbus (Fig. 1, A), a preplaced 6-0 black silk suture positioned (Fig. 1, B), and a corneal section made with a cataract knife (Fig. 1, C). Lengths of polyethylene tubing were cut to measure 5 mm. longer than the circumference of the cornea, bent in half (Fig. 1, D), and inserted into the anterior chamber through the incision (Fig. 1, E). The elasticity of the tubing caused it to spring into a circular position so that the tubing pressed quite firmly into the angle of the anterior chamber. The preplaced suture was tied and cut, and the anterior chamber re-formed with a small bubble of air injected through the incision (Fig. 1, F). The second eye served as a control.

In 3 rabbits that developed elevated intraocular pressure in the operated eye, a 15 cm. length of the polyethylene tubing was positioned in the eye 5 days after operation so that one
opened end was in the anterior chamber and the other sealed end came out to the skin surface above the orbit as described elsewhere.\textsuperscript{3} (The polyethylene tube will be referred to as the longitudinal tube as opposed to the circular tube within the angle of the anterior chamber.)

The intraocular pressure was measured at weekly intervals either by manometry via the longitudinal tube\textsuperscript{3} or by Schiotz tonometry (1955 calibration tables). All rabbits were killed after 3 months, the eyes sectioned and stained with hematoxylin and cosin or Loyez iron hematoxylin for myelin. Chemical determinations for ascorbic acid and protein in the aqueous humor were performed as previously reported.\textsuperscript{3}

Results

Elevated intraocular pressure (50 to 60 mm. Hg) and corneal haze occurred within 24 hours after the circular tube implantation in 6 of 10 rabbits. If the pressure was not elevated within this period of time, it remained normal throughout the experiment. (The animals were killed after 2 months.) The 3 rabbits with elevated intraocular pressure which did not have longitudinal tubes inserted into the anterior chamber maintained a pressure between

Fig. 1. Operative technique for implantation of circular polyethylene tubing in the anterior chamber angle.

Fig. 2. High-power view demonstrating fibrosis and disruption of spaces of Fontna by implantation of circular tube.
40 and 50 mm. Hg for about 2 months; it slowly decreased to a level of 30 to 35 mm. Hg. During the 3 month period, the corneal diameter increased from an average of 12 to 15 mm., the cornea remained hazy, and the pupil became dilated and fixed to light. Despite corneal haze, there was little corneal vascularization except in the area of the corneal incision. Moderate conjunctival and circumcorneal injection was present.

The 3 rabbits with elevated intraocular pressure in which longitudinal tubes were inserted into the anterior chamber provided additional data. Direct measurements of intraocular pressure by manometry through the longitudinal tube verified intraocular pressures of 40 to 45 mm. Hg during the 3 weeks following the initial surgical procedure. Chemical determinations of the aqueous humor indicated an increase in the permeability of the blood-aqueous barrier, a reduction in the ascorbic acid concentration of 15 to 20 mg. per cent (normal 25 to 30 mg. per cent), and an elevation in the protein concentration of 1,000 to 1,500 mg. per cent (normal 15 to 150 mg. per cent). Because of the excess

---

Fig. 3. A, Optic nerve of normal control eye. (Hematoxylin and eosin.) B, Optic nerve of normal control eye. Note deep "physiological" cupping. (Hematoxylin and eosin.)
protein, the longitudinal tubes became occluded about 3 weeks after insertion, and measurements of intraocular pressure were continued by tonometry.

That the elevation in intraocular pressure was related to blockage of aqueous humor outflow channels was demonstrated in rabbit No. K-79 as follows: Ten days after implantation of the circular tube and 5 days after insertion of the longitudinal tube the intraocular pressure was 48 mm. Hg and the cornea was quite hazy, obscuring details of the anterior chamber. At this time the sealed end of the longitudinal tube was partially opened so that aqueous humor drained from the eye through the tube at a flow rate of about 3 c.mm. per minute. The pressure slowly fell to 20 mm. Hg; two days later the corneal haze had cleared and the inserted tubes were visible. At this time the tip of the longitudinal tube was sealed again. Following this, the intraocular pressure rose to 42 mm. Hg; two days later the cornea was hazy once again. Upon reopening the sealed end of the longitudinal tube the pressure fell, the cornea cleared, the details of the anterior chamber were visible and remained so for the next 11 days during which time aqueous humor drained from the eye through the tube. Sealing the end of the longitudinal tube caused elevation of pressure once again and it remained so for the balance of the 3 months' period.

Histologic examination of the eyes with elevated intraocular pressure demonstrated the circular tube in position with little cellular reaction other than fibrosis in the angle of the anterior chamber. The tension of the circular tube had pushed it several millimeters into the ciliary body and the spaces of Fontana and the venous outflow channels were obliterated by fibrosis (Fig. 2).

Posteriorly, the optic nerve of the normal control eye presented several variations of depth of optic nerve head (Figs. 3A and 3B), but in all cases the optic nerve fibers were normally myelinated. In contrast, the optic nerve head of the eyes with elevated

Fig. 4. Appearance of nerve head after 3 months of elevated intraocular pressure. (Hematoxylin and eosin.)
intraocular pressure were markedly excavated (Fig. 4), and there was loss of myelin from much of the optic nerve. Loss of retinal ganglion cells with relatively good preservation of the remainder of the retina was noted in the eyes (Fig. 5A) when compared with the opposite normal control retinas (Fig. 5B).

Discussion

Huggert\(^2\) has described several techniques for blocking aqueous outflow. The present technique conforms to the general rule of provoking a rapid development of a sustained pressure rise within 24 hours. Whether the late gradual decrease in intraocular pressure is a result of decreased aqueous formation could not be determined with any certainty in this preparation in which the blood-aqueous barrier was no longer intact.

By intermittently draining aqueous humor through the longitudinal tube, the reversibility of the corneal haze and intraocular pressure rise is amply demonstrated. This would suggest that most, if not all,
of the outflow channels in the anterior chamber angle are obstructed.

Although histologic examination of the retina and optic nerve of rabbits with congenital buphthalmos and increased intraocular pressure has indicated no particular pathologic changes,4,5 there has been no similar study in experimentally induced elevation of intraocular pressure. In the present study, retinal ganglion cells were markedly reduced in number and there was excavation of the optic nerve head with partial loss of myelin after 3 months of elevated intraocular pressure. From clinical data, this is what one would expect, and, in this regard, it is noteworthy that these specific histopathologic changes occurred in association with an increased intraocular pressure. Such selective changes are in contradistinction to the extensive retinal degeneration in the rabbits of Flocks and co-workers6 which was, in all probability, a result of vascular occlusion secondary to the sudden high elevation of intraocular pressure. Of peripheral significance is the "cupped" appearance of the normal rabbit optic nerve head (Fig. 3B); it appears flat only when sectioned through that portion from which the bundle of myelinated nerve fibers arises (Fig. 3A). One needs the additional verification of loss of myelin from the degenerated optic nerve fibers before "cupping" of the nerve head is considered pathologic.

Of interest is the recent report of failure to produce an elevation of the intraocular pressure by inserting a circular polyethylene tube in the chamber angle.7 It is difficult to determine why the intraocular pressure was not elevated without histologic evidence of compression of the trabecular meshwork and venous outflow channels by the tube.

Addendum

Since this paper was submitted, another study of retina and optic nerve with experimentally increased intraocular pressure in the rabbit has been reported.5

REFERENCES