In our experimental animals, the findings support those of previous workers. Necrosis of the RPE and outer retina were prominent acute features. In areas of moderate to heavy treatment, there was partial disappearance of the choriocapillaris within 2 weeks. A diffuse HRP leakage was seen in the edematous choroid, concentrated around the choriocapillaris and Bruch's membrane.

A firm chorioretinal adhesion characterized by intercellular junctions and villous interdigitation of glial cells and regenerating RPE was present by 1 month. Much of the choriocapillaris was obliterated. In some areas HRP was undetectable. In others, leakage extended to the level of intercellular junctions between adjacent Müller cells. Choroidal edema had subsided. From 2 to 6 months, glial proliferation and junctional complexes increased in prominence, basal lamina and collagen deposits appeared, and pigment-laden cells were seen in the inner retina and vitreous. There was no regeneration of the choriocapillaris or change in the pattern of HRP leakage.

The blood-retinal and chorioretinal barriers are not normally permeable to HRP, but may be broken by a variety of stimuli including thermal injuries. Leakage of HRP into the outer retina after cryotherapy was expected. The retinal vessels in the treated areas were not the source of leakage. Edema of the choroid is commonly seen in the acute phase of healing following chorioretinal cryotherapy, which presumably damages the choriocapillaris and RPE. Although HRP is capable of leaking from the normal choriocapillaris, the RPE is impermeable to the tracer. Permanent injury to the RPE demonstrated up to 6 months accounts for persistence of HRP leakage. The absence of HRP in heavily treated areas is explained by the absence of choriocapillaris. The normal passage of HRP, with a molecular weight of 40,000, through structurally altered outer retina demonstrates a permanent change in the chorioretinal barrier and could account for fluorescent leakage through a cryogenic chorioretinal scar.

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Fig. 1. Light micrograph of the cross-sectioned optic nerve of nontreated dog. (×430.)

Fig. 2. Light micrograph showing the optic nerve of the high-dosage group (15 mg./kg./day). The reduced nerve fibers with more increased space among the axons are quite obvious. (×430.) Inset: Electron micrograph showing two axons with thin myelin sheath. Arrow indicates degenerative axon. (×3,800.)

Retina, has been reported in rats treated with fenthion. The histopathology of the retina and the optic nerve in the course of chronic OP intoxication, however, does not appear to have been studied previously.

In order to evaluate the chronic toxic effect of OP on the retina and the optic nerve, beagle dogs received for 2 years orally administered ethylthiometon (diethyl S(2-ethylthioethyl) phosphorothiolothionate). After the termination of the treatment, both histopathological and electron microscopic observations were carried out.

Materials and methods. Ten adult beagle dogs, 6 months old and weighing 5 to 10.6 kg., were used. Detailed descriptions of the dogs have been published elsewhere. Animals were orally treated with gelatine capsules containing ethylthiometon corresponding to about 1/100 of its lethal dosage level of 50 mg./kg. Treated animals consisted of three groups each, to which were administered doses of approximately 0.5 (low), 1.0 (middle), and 1.5 mg./kg./day (high dosage) for 5 days/week for 2 years, when the weight of the dog was assumed to be about 10 kg. Empty capsules were given to the control dogs.

One week after the termination of the adminis-
Fig. 3. Electron micrograph of the retinal pigment epithelial cells (PE) of a treated dog of low-dosage group at the peripapillary portion. The cytoplasm is filled with a mass of unusual lamellar structures and pigment granules. (×7,800.)

Fig. 4. Electron micrograph of the peripheral portion of the retina of the treated dog. Dense bodies (db) were seen in the outermost cytoplasm of Müller cells (M) near the outer limiting membrane (OLM). IS, Inner segment of visual cells. (×4,800.) Inset: Higher magnification of the dense bodies. Many glycogen particles were seen associated with them. (×28,000.)

istration, the animals were anesthetized by intravenous injection of pentobarbital sodium (35 mg/kg.). The eyes were extirpated and immediately placed in 4 percent glutaraldehyde in 0.1M phosphate buffer for 2 hr. The retina was sampled in series from the peripapillary region to the ora serrata, in both the nasal and temporal quadrants of the eye. The remaining retinal tissues were divided into tapetal and nontapetal areas and then subdivided into small pieces. The optic nerve sample was obtained from a portion of about 5 mm behind the eyeball. All tissue was postfixed in 1 percent osmium tetroxide for 2 hr., dehydrated in ethanol, and embedded in Epon 812. Semi-thin sections were studied with the light microscope, and ultrathin sections were examined with a Hitachi HU-12 A electron microscope.

Results

Optic nerve of control animals. The central portion of the optic nerve of the control dogs contained many fasciculi divided by delicate
processes of the astrocytes. Each fasciculus consisted of numerous myelinated fibers ranging from 0.5 to 9 μm in diameter (Fig. 1). The round or sometimes indented nuclei of the astrocytes, were usually observed at the margin of the fasciculi, but occasionally some were seen between them. Dark, ovoid cells, identified as oligodendrocytes, were often arranged among the myelinated axons.

**Optic nerve of treated animals.** Pathological changes of the optic nerve in the treated animals consisted of reduced number of nerve fibers combined with the proliferation of glial cells. Changes of the optic nerve in the low-dosage group were relatively milder than that of the middle- or high-dosage groups. Necrotic masses of myelin sheath were only rarely found.

The morphological appearance in the middledosage group was basically similar to that of the high-dosage group. Severe destructive change was seen in the high-dosage group (Fig. 2). Myelin sheaths of surviving axons frequently became very thin (Fig. 2, inset). Some of them formed concentric lamellae, suggesting the process of degeneration.

**Retina of treated animals.** The retinas of all treated dogs were distinguishable histologically by partial necroses of the pigment epithelial cells as well as of the Müller cells. The pigment epithelial cells at the peripapillary portion of the retina were constantly affected. The most peripheral portion escaped this damage.

The cytoplasm of the affected cells lost their characteristic reticulated appearance at the involved portion and became lamel late near the pigment granules (Fig. 3) or severely vacuolated. The last-named finding was the most dominant feature. At higher magnification the lamellar structures were distinctly seen to be in continuity with cisternae of smooth endoplasmic reticulum. Mitochondria appeared intact in these groups.

Müller cells located at the peripheral portion of the retina approximately 2 or 3 mm. posteriorly from the ora serrata showed pathological changes at the outer portion of their elongated cytoplasm, which contained several unusual dense bodies ranging from 0.4 to 0.8 μm in diameter (Fig. 4). Occasionally they were associated with glycogen particles (Fig. 4, inset). The higher the dosage, the more severe changes were noted.

These findings were seen in all the treated dogs, regardless of dosage levels. They were never observed in the control animals.

**Discussion.** The present study demonstrated that long-term administration of the OP induced distinct histopathological changes in both optic nerve and retina in all treated dogs, although the lesion in the retina was mild. In addition, the nerve fibers in the optic nerve showed conspicuous reduction of the number of the fibers, which was to some extent in proportion to the applied dosage. Since the above changes were not seen in the control animals, they were considered to be due to the OP. These experimental results may demonstrate a causative factor in the production of a reduced visual acuity and a constriction of the visual field, which were frequently seen in clinical cases with chronic OP intoxication.1-3

The mechanism that the OP with cholinesterase-inhibiting action induced optic atrophy was unknown, but the histological appearance of axonal degeneration may be the result of direct toxic action of the OP itself or of its metabolites on the neurotrophism. Since cell bodies of ganglion cells in the retina and of oligodendrocytes in the optic nerve remained intact, the OP may affect the axoplasmic transport occurring within the nerve fibers of the optic nerve. Ochs4 stated that axoplasmic transport is blocked not only by anoxia but by metabolic inhibitors.

The pathological changes in retinal ganglion cells, which were suggested to be secondary to the axonal lesion of the optic nerve, were not seen. To a small extent, the pigment epithelium at the peripapillary region as well as the Müller cells at the peripheral retina showed pathological changes. The former seems to be a result of the endematous optic disc rather than the toxic action of the OP, since the lesion was located at an extremely limited area. Edema of the optic disc with enlarged blind spot is known to exist in clinical cases of OP intoxication.3 The pathological effects involving the peripheral retina may be related to an abnormal toxic effect of the OP, since the dense bodies seem to be emerging either from degenerative smooth endoplasmic reticulum or from aggregated glycogen particles. Moreover, variation of the damaged portions between peripapillary and peripheral regions of the retina may be due to different dependency of the retinal nutrition on either choroidal or retinal vessels.

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