

## Radiation retinopathy: electron microscopy of retina and optic nerve

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**Summary.** A 4 1/2 year old female was treated for embryonal rhabdomyosarcoma of the left orbit in 1975 with radiation (59.5 Gy in 5 weeks), followed by chemotherapy. An electroretinogram (ERG) in March, 1988 revealed cone responses 3% of normal and no rod responses in the left eye, and normal responses in the right eye. The eye was enucleated in April 1988. In the fovea no choroidocapillaris was seen at the intact Bruch's membrane, and the pigment epithelium was preserved only in small patches. No photoreceptor cells were seen in the areas devoid of pigment epithelial cells. The parafoveal and peripheral (30° eccentricity) retina was better preserved. The thickness of the layer of rods and cones and of Henle's fiber layer was reduced. Very few outer segments were present. Macrophages had invaded the retinal tissue in moderate numbers. The retinal vessels were ensheathed by several layers of collagen fibrils. The spatial densities of pigment epithelial, cone, rod, and bipolar cells had been reduced. The optic nerve contained a total number of 1,022,000 nerve fibers.

**Key words:** Chemotherapy, Radiation therapy, Human choroid, Human optic nerve, Human retina

### Introduction

Retina and optic nerve can tolerate relatively high doses of ionizing radiation. Exposure of up to 50 Gy (1 Gy = 100 rad) in divided doses does not lead to radiation damage to the retina in most individuals (Haik et al., 1983). However, cases of radiation retinopathy have been reported after doses of 35 to 50 Gy (Wara et al., 1979; Brown et al., 1982; Haik et al., 1983). It may

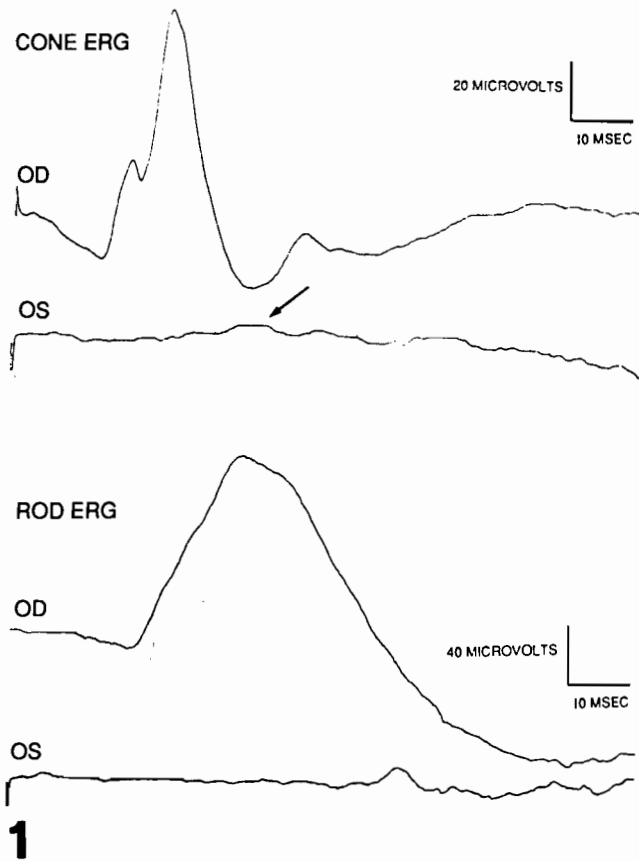
develop relatively late, sometimes many years, after exposure of the retina to the radiation (Merriam et al., 1972). Clinically the disorder is recognized by the delayed appearance of ischemic vascular changes similar to diabetic retinopathy (Hayreh, 1970). The histopathology of radiation retinopathy has been reported (Cibis and Brown, 1955; Perrers-Taylor et al., 1965; Howard, 1966; Hayreh, 1970; Brown et al., 1982; Parsons et al., 1983). Reeser et al. (1978) reported fine structural changes in a human retina a few weeks after exposure to 300 Gy. In this study, it was ambiguous if the loss of photoreceptor outer segments, the fatty degeneration of pigment epithelial cells, and the invasion of the retina with macrophages had resulted from the irradiation or if these changes had been the reaction to the primary lesion, a retinal hamartoma. We have studied by electron microscopy the retina and optic nerve of a patient whose eye was enucleated 13 years after radiation treatment for orbital rhabdomyosarcoma.

### Materials and methods

#### Case history

A 4 1/2 year old girl was treated for embryonal rhabdomyosarcoma of the left orbit in 1975. The left orbit was irradiated with 50 Gy in 5 weeks (2 Gy daily) from a cobalt-60 source via an anterior 5 x 5 cm field, followed by 9.5 Gy delivered in divided doses over 5 days via lateral 4 x 4 cm fields with a 6 MeV linear accelerator. Radiation therapy was completed on Jan. 26, 1976. Combination chemotherapy was begun on December 16, 1975. Vincristine was given intravenously at a weekly dose of 2 mg/m<sup>2</sup> for 12 weeks. At week 13 cytoxan was given daily intravenously for five days and then per os for seven days at a dose of 10 mg/kg, and thereafter 2.5 mg/kg cytoxan was given daily p.o. up to 24 months. Actinomycin D was given intravenously at a daily dose

Radiation Retinopathy

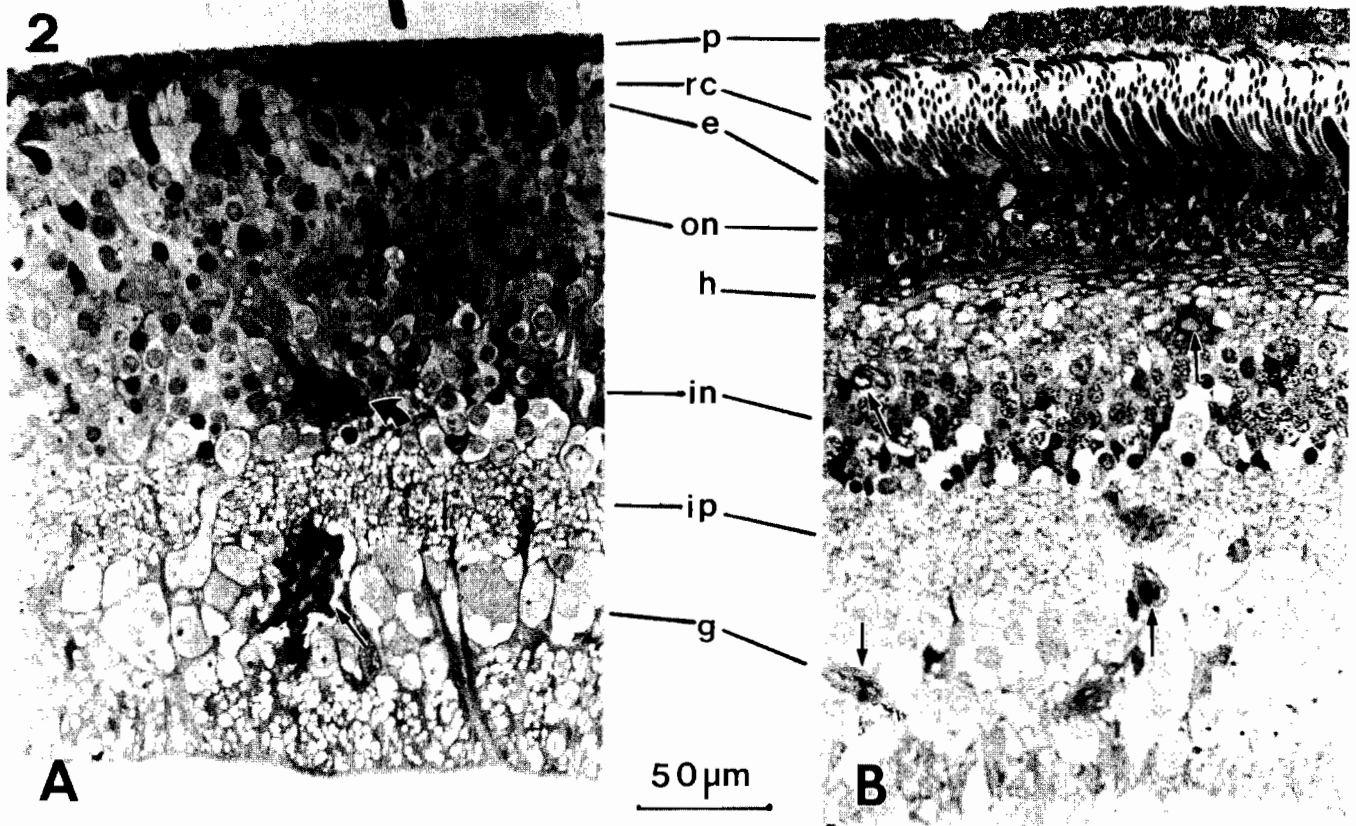


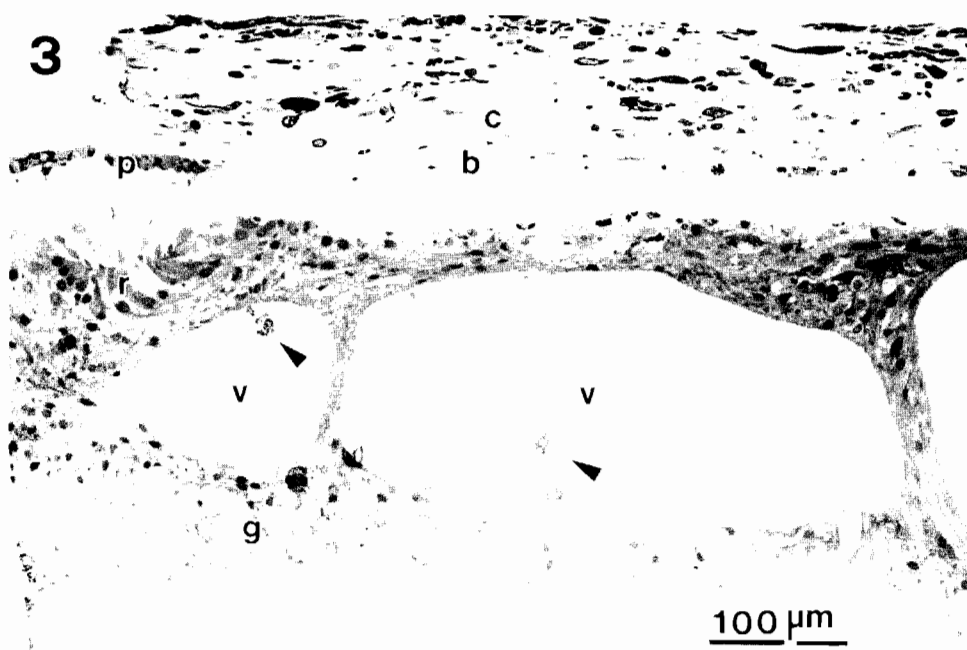
of 0.015 mg/kg for five days at weeks 1, 18, 30, 42 and 52. Periodic systemic evaluation for metastases were negative.

In January, 1978 a small posterior subcapsular opacity of the left lense was detected. It became a dense cataract in June, 1978. In September, 1979 a large left exotropia and hypertropia developed. The patient continued to complain of intermittent pain. In March, 1988 the intraocular pressure was 12 mm Hg OD and 14 mm Hg OS. Exophthalmometry readings were 18 mm OD and 10 mm OS. A dense vascular pannus covered the left cornea. Tear production was reduced in the painful left eye. On March 28, 1988 an electroretinogram (ERG) revealed cone responses which were 3% of normal. Rod responses were not detected in the left eye. The responses in the right eye were normal (Fig. 1). Vision in the right eye was 20/15. On April 18,

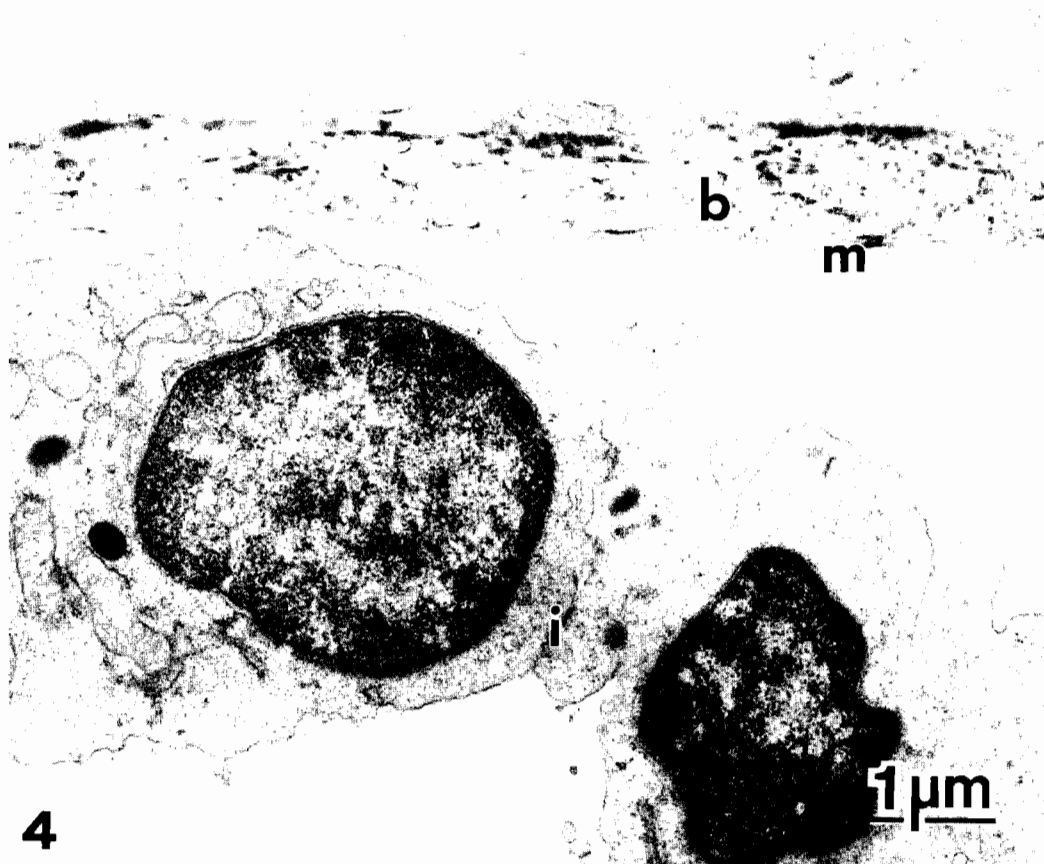
**Fig. 1.** Cone (above) and rod (below) electroretinograms from the normal (OD) and the irradiated (OS) eye. The calibrations on the right indicate the amplitude and time scale of the responses. The arrow points to the small cone response detectable from the abnormal eye.

**Fig. 2.** Light micrographs of semithin sections of the parafoveal retina of the irradiated and of the untreated eye. The layer of rods and cones is dramatically reduced in thickness and Henle's fiber layer is not detectable in the irradiated retina. p - pigment epithelium; rc - layer of rods and cones; e - external limiting membrane; on - outer nuclear layer; in - inner nuclear layer; ip - inner plexiform layer; g - ganglion cell layer; arrows - blood vessels; curved arrow - blood vessel a section of which is shown in Fig. 9.  $\times 620$

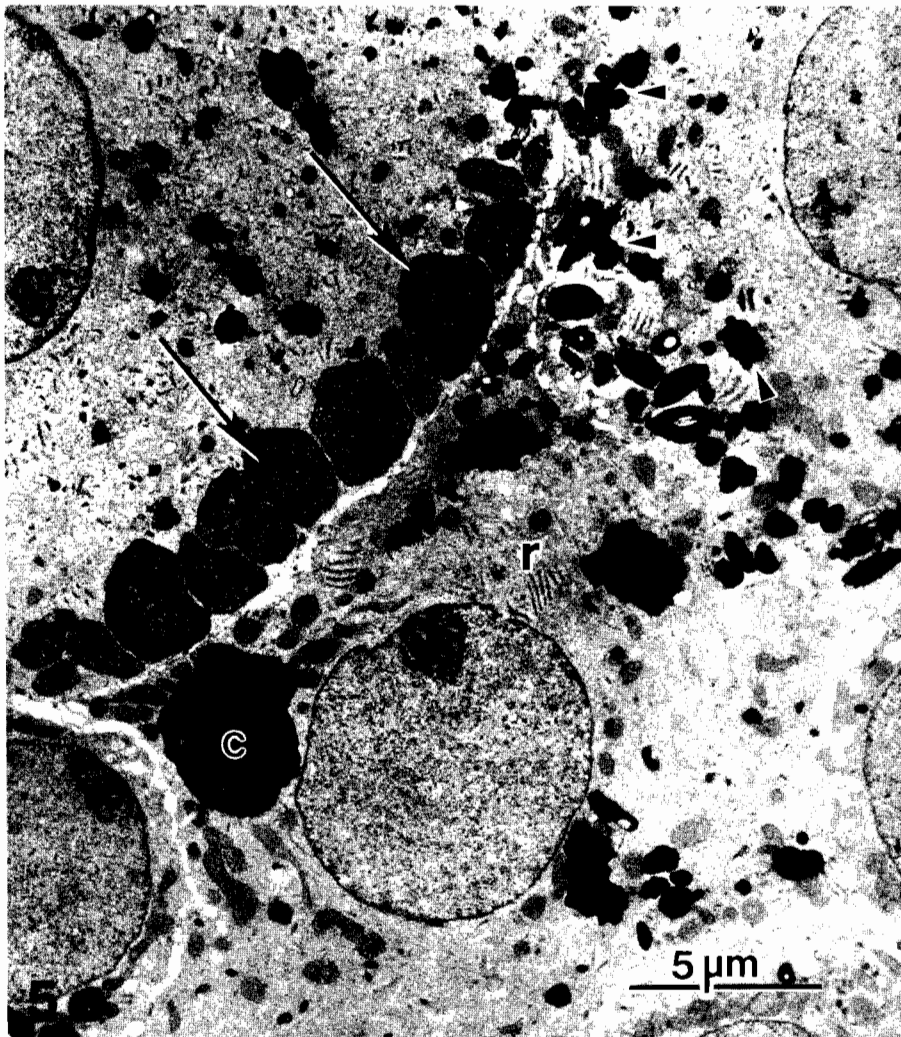




**Fig. 3.** Light micrographs of a semithin section through the fovea. c - choroid; b - Bruch's membrane; p - pigment epithelium; r - photoreceptor cells; v - vacuole; g - ganglion cells. Arrowhead points to macrophage.  $\times 180$



**Fig. 4.** Electron micrograph of a section of the fovea showing the denuded Bruch's membrane (b) and some cells, loosely attached to it. Junctional complexes (i) are seen between the cells. m - basilar membrane.  $\times 16,640$



**Fig. 5.** Tangential section through the pigment epithelium. Pigment granules (arrowheads) display various sizes and shapes. Abnormal mitochondria (arrows) are seen in some pigment epithelial cells. c - composite melanosome; r - small stacks of rough endoplasmic reticulum.  $\times 5,000$

1988, an enucleation of the left eye was performed under general endotracheal anesthesia.

#### *Electroretinography*

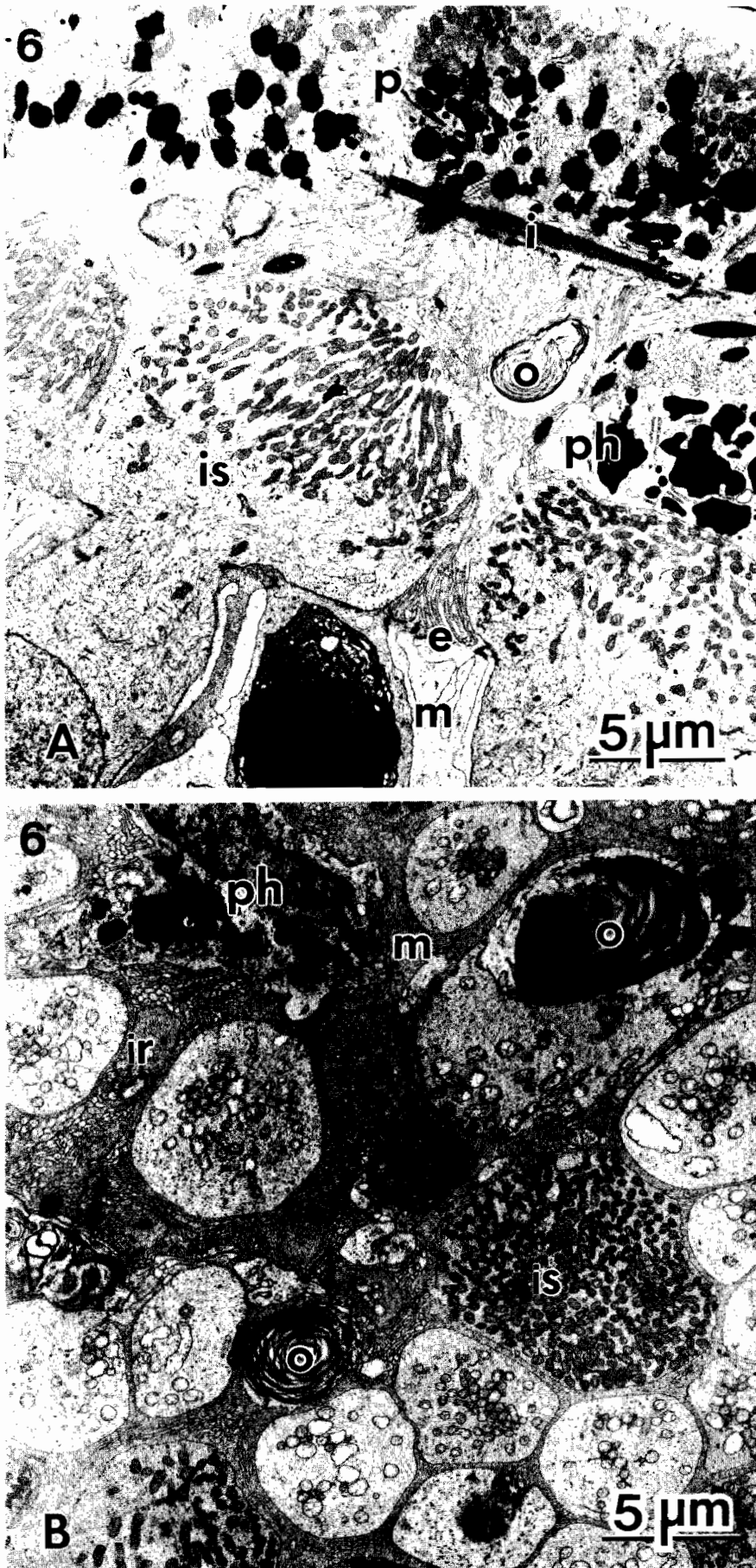
The electroretinography was performed on both eyes using Burian Allen contact lenses, corneal anesthesia and fully dilated pupils. The stimulus was a ganzfeld system, fully described in Gouras and MacKay (1989).

#### *Light and electron microscopy*

The eye was fixed by immediate immersion into 2% glutaraldehyde and 2% formaldehyde buffered with 150 mMol cacodylate to pH 7.4. The retina was sampled at three loci. The first one was in the nasal quadrant at the same distance as the fovea from the optic nerve. This locus is referred to as peripheral retina. It corresponds to  $30^\circ$  eccentricity. The second locus was at the parafoveal areas 1.5 mm from the foveolar center, and the third contained the fovea. The specimens were embedded so that either sections

parallel to a plane tangential to the surface of the globe (tangential sections) or radial sections could be cut. Semithin sections (0.5 to 1  $\mu\text{m}$ ), stained with toluidine blue and basic fuchsin, were used for light microscopy. Ultrathin sections were stained with uranyl acetate and lead citrate. Cellular densities were determined in composite electron micrographs of tangential sections taken from the appropriate retinal layers. The details of the morphometric method have been described elsewhere (Krebs and Krebs, 1987, 1991). The method allows accurate determination of spatial cell densities in the vertebrate retina. Cell counts within any one sample deviate from each other by less than 5% (Krebs et al., 1990).

A 2 mm thick slice was cut from the optic nerve close to its entrance into the sclera. The slice was embedded in epoxy resin such that semithin sections could be cut which represented complete cross sections of the nerve. The same block which yielded the semithin sections was retrimmed to allow ultrathin sectioning of a third of the nerve's cross section containing the central vessels. Electron micrographs



**Fig. 6.** Layer of rods and cones in radial (A) and tangential (B) sections. p - pigment epithelium; i - junctional complex; ph - phagocyte; o - outer segment material; is - inner segment of cone; ir - inner segment of rod; e - external limiting membrane; m - radial glial (Müller) cell cytoplasm.  $\times 4,200$

were taken covering 100 by 100  $\mu\text{m}$  fields in the peripheral and central parts of the segment. The total cross sectional area of the nerve was determined in micrographs of the semithin sections. The area of the connective tissue septa and of the central vascular domain was subtracted from the total cross sectional area, in order to obtain the total area of cross sectioned nerve fascicles (neural area). The number of cross sectioned nerve fibers per  $\text{mm}^2$  within the fascicles was determined in electron micrographs of 3,150 fold magnification. The total number of nerve fibers was calculated separately for the central and peripheral part of the nerve, using the neural area determined in the light micrographs and the fiber densities measured in the electron micrographs. The ratio of areas of axonal profiles versus interstitium was measured in electron micrographs of 8,600 fold magnification. The thickness of the myelin sheaths of axons was measured in the same micrographs, using about 100 randomly selected axons at locations at which the myelin sheath was sectioned perpendicularly. A computerized image analysis system (Sigmascan<sup>®</sup>) was used. For comparison retina and optic nerve of a 58 year old man who died of Hodgkin's disease was studied in the same fashion. The total cross sectional area of this nerve could not be determined because it had been embedded in small pieces. About two thirds of the nerve, including the central vessels, were available for analysis.

## Results

### Electroretinography

The ERG of the patient, one month before enucleation, is shown in Fig. 1. The upper traces show the ganzfeld response of the cone system obtained in the presence of a bright ganzfeld adaptation field. There is a small response, approximately 3% of



Fig. 7. Radial section through the outer nuclear layer. A photoreceptor cell displays fatty degeneration (asterisk). ph - phagocyte; m - radial glial (Müller) cell cytoplasm.  $\times 4,200$

that obtained from the right eye, which is well within normal limits. There is no evidence for any rod activity from the left eye (lower trace).

#### Structure of retina

On inspection of the fixed retina, the macula had a dull gray color and no foveal pit was seen. The rest of the retina was grossly normal. No macroscopic evidence of prior retinal detachment was detected. The spatial densities of the retinal cells are summarized in Table 1.

Semithin sections revealed that the layer of Henle's fibers and the layer of rods and cones were most affected in the treated retina. The width of the layer of rods and cones was reduced to  $5 \mu\text{m}$ , and Henle's fiber layer was absent. The remaining layers of the

irradiated retina were almost as thick as in a normal retina (Fig. 2).

The structure of the central fovea was destroyed. Large empty cysts were seen in the foveal neuroretina (Fig. 3), and the choroidocapillaris was absent. Large patches of Bruch's membrane were not covered with pigment epithelium (Figs. 3, 4). In these regions no cones were found. Cones were detected only where pigment epithelial cells were present. Isolated strings of cells attached to each other by junctional contacts but devoid of pigment granules were present in the subretinal space of the fovea (Fig. 4).

In the parafoveal and peripheral retina, the pigment epithelial cells numbered 4,350 and 2,700 per  $\text{mm}^2$  (Table 1). Myelin-bodies and small stacks of rough endoplasmic reticulum were seen in the cytoplasm (Fig. 5). Binucleated pigment epithelial cells

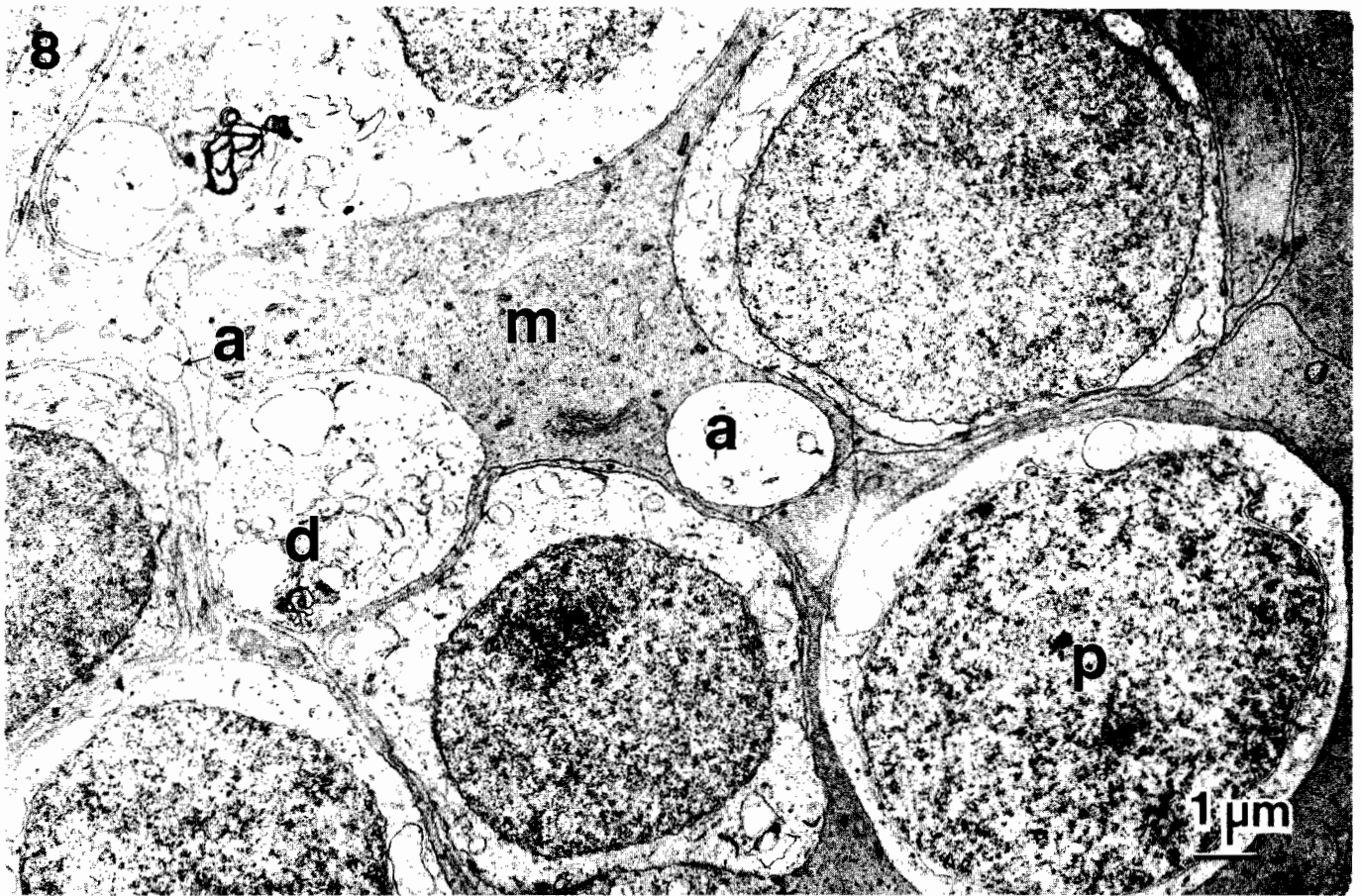


Fig. 8. Tangential section through the inner nuclear layer. Perikarya (p), axonal (a) and dendritic (d) fibers of the bipolar cells are embedded in a matrix of radial glial (Müller) cells (m).  $\times 8,200$

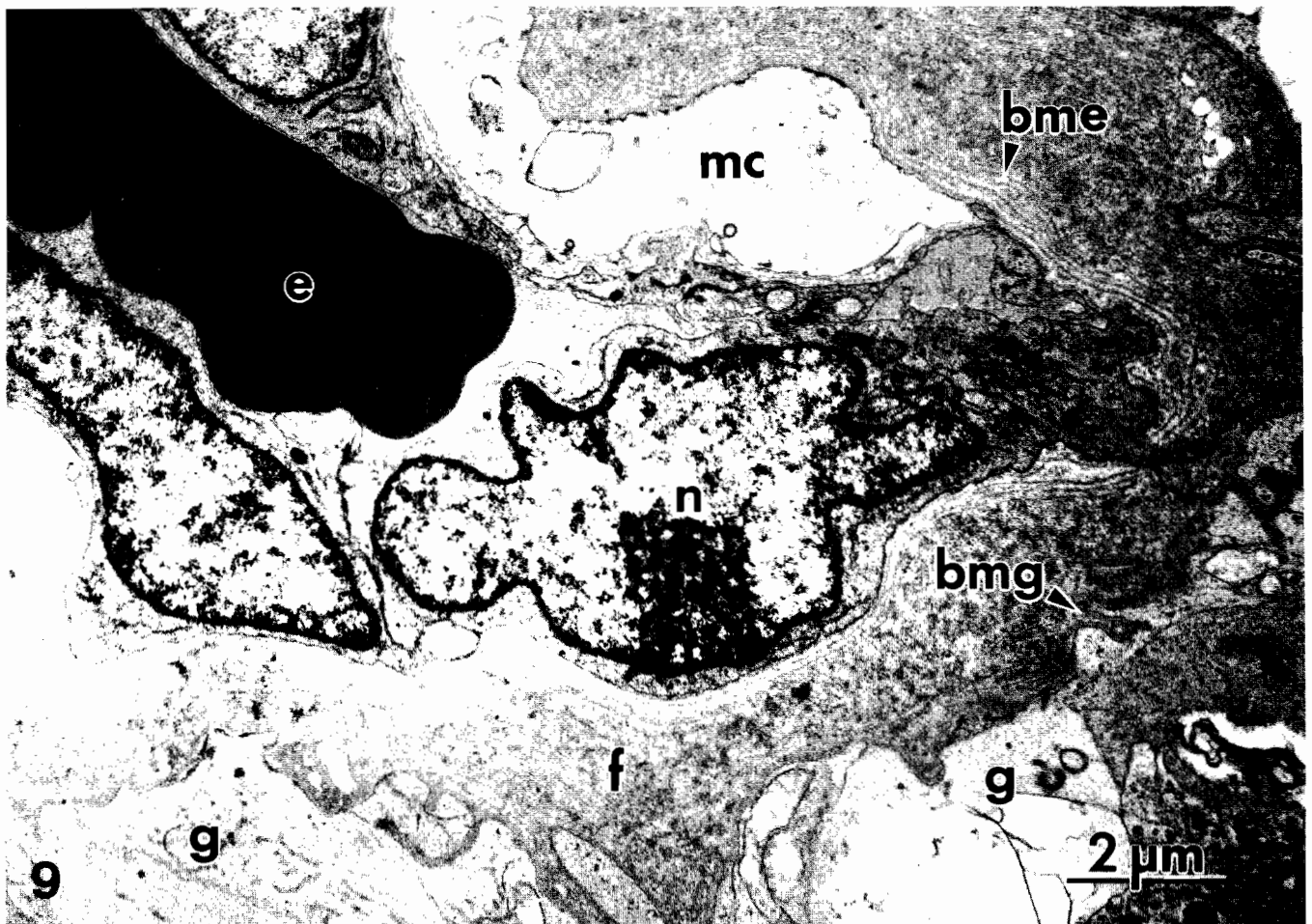
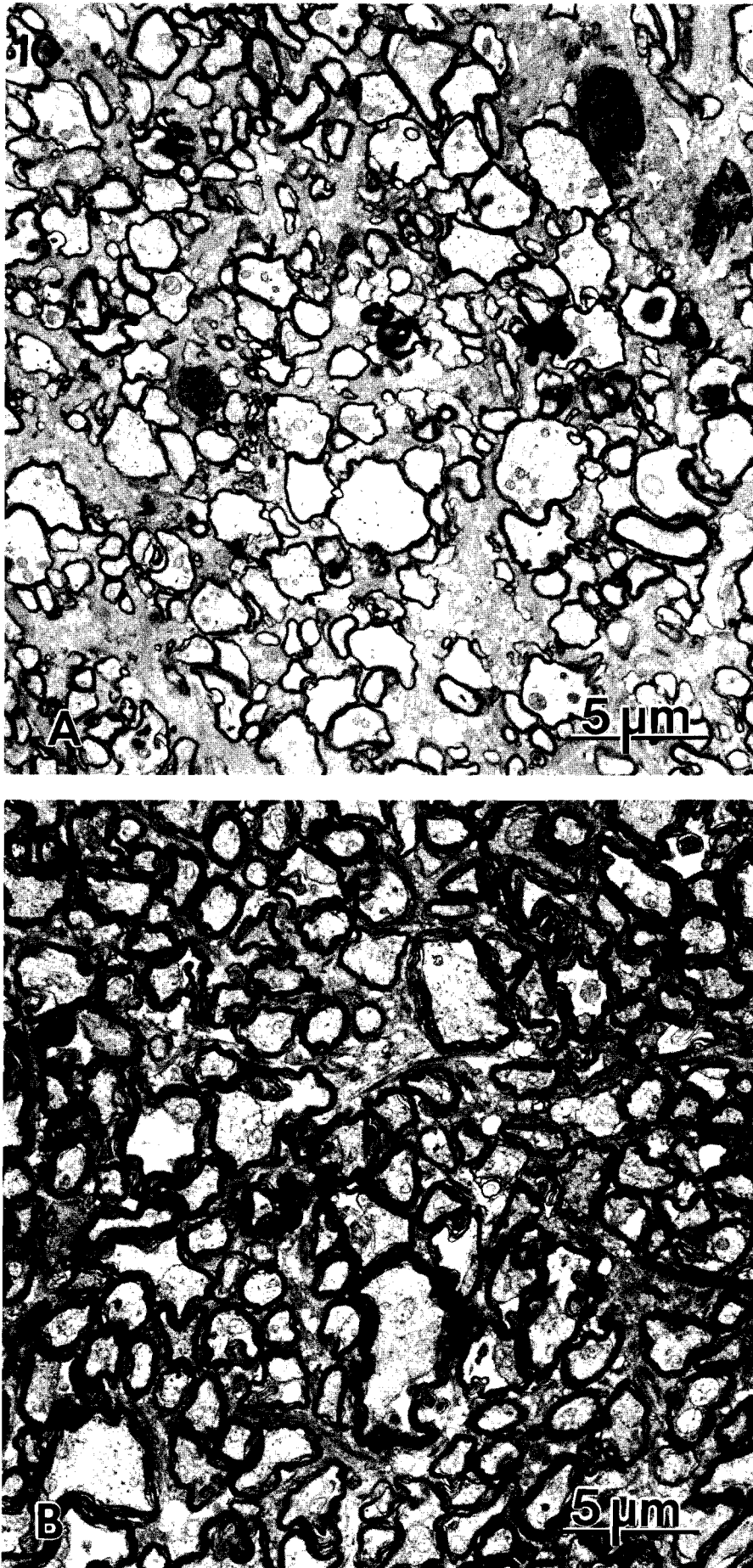


Fig. 9. A blood vessel in the inner nuclear layer (Fig. 2, curved arrow). bme - multilayered basilar membrane of the endothelial cells; bmg - basilar membrane of radial glial cells; e - erythrocyte; f - thick fibrous layer; g - extensions of radial glial (Müller) cells; n - nucleus in an endothelial cell; mc - smooth muscle cell.  $\times 11,600$



**Fig. 10.** Cross sections through the optic nerves of the irradiated 17 year old patient (A) and of the non-irradiated 58 year old male (B)  $\times 4,200$

were encountered occasionally. The pigment granules varied considerably in shape and size. Lysosomes in various stages were abundant. In some pigment epithelial cells bizarre and enlarged mitochondria were seen (Fig. 5).

Outer segments were rarely seen in radial sections (Fig. 6A). Tangential sections revealed that some outer segments and connecting cilia were present (Fig. 6B). Henle's fiber layer was absent in the parafoveal region. The photoreceptor synapses contained synaptic ribbons. Flat or conventional synapses were seen too. Some degenerating photoreceptor cells, often filled with lipid droplets (Fig. 7), lay in the outer nuclear layer. Occasional phagocytes were encountered between the retinal cells.

The remaining retinal layers in the peripheral and parafoveal samples were not reduced in width because the cell bodies of surviving cells had increased in size, and radial glial (Müller) cells filled the spaces between the retinal neurons. No glial scars were found (Fig. 8).

Retinal blood vessels were conspicuous for a thick sheath of dense collagen bundles (Figs. 2, 9). Radial glial (Müller) cells contacted the fibrous ensheathment of the retinal vessels (Fig. 9). The fibrous sheath was between the frequently multilayered basilar membrane of endothelial cells and the simple basilar membrane of the radial glia (Fig. 9). The capillary beds, normally present at the levels of horizontal and amacrine cells, were reduced to a few mainly unbranched capillaries. Blood vessels of the choroid did not show similar pathologic changes.

#### *Optic nerve*

The irradiated nerve contained 1,022,000 axons. More connective tissue was present between its axons than in the unirradiated nerve (Fig. 10) of a 58 year old man. Table 2 summarizes the morphometric



findings in both nerves. Some macrophages, often containing composite lysosomes with myeloid inclusions, were found in the irradiated nerve. A few degenerated axons were seen in both nerves. The blood vessels appeared normal in the irradiated nerve. Extravascular erythrocytes were found in the connective tissue close to the central artery in the treated nerve. Since a complete cross section of the non-irradiated nerve was not available, the total number of nerve fibers could not be determined.

**Table 1.** Cellular densities (cells/mm<sup>2</sup>) of the irradiated retina.

	periphery*	parafovea <sup>+</sup>
Pigment epithelial cells	2,722	4,348
cones	3,789	5,238
rods	34,316	31,333
Bipolar cells	34,869	67,007

\* retina at 30° nasal eccentricity; <sup>+</sup> retina at 1,000 μm from the foveolar center.

**Table 2.** Morphometric data of the optic nerves of the irradiated and of an untreated eye.

	irradiated		untreated	
	inner	outer	inner	outer
connective tissue*	30.8%	17.7%	22.8%	14.2%
Axons/mm <sup>2</sup> +	267,337	301,933	199,168	169,234
Axon area	53.14%	57.06%	73.13%	68.22%
Myelin sheat diameter	90 (± 30)nm	150 (± 40)nm	420 (± 70)nm	310(± 90)nm

The values are taken from the inner and outer 50% of the cross section of the nerves. The untreated nerve is from a 58 year old man who died of Hodgkin's disease.

\* Relative area of connective tissue septa between the fascicles in the cross section of the nerves; The values for the inner half include the central vessels.

+ Axon density within cross sections of the fascicles. Relative area of axon profiles within cross sections of fascicles. The diameter of the myelin sheath of 100 randomly selected axons; the numbers in brackets are the standard deviations.

## Discussion

Studies in primates (Cibis et al., 1955) indicate that rods are more susceptible to radiation damage than cones. Accordingly, rod cells were damaged more than the cone cells in the patient. Compared to Osterberg's (1935) and Curcio et al. (1987) data, the spatial density of cones was reduced by approximately 50% in the parafovea and by approximately 40% in the periphery. Rod density had decreased by about 65% and 70% (Table 1). The ERG recordings (Fig. 1) revealed absence of a rod response and a very weak cone response because most photoreceptor cells had lost their outer segments. Radiation, possibly enhanced by chemotherapy, affected the ability of the remaining photoreceptor cells to maintain or to regenerate outer segments.

The non-irradiated 58-year old man, whose optic

nerve is used in Table 2, had 6,000 pigment epithelial cells per mm<sup>2</sup> in the fovea. The number of these cells was significantly lower in the patient (Table 1). Binucleated pigment epithelial cells occur in normal human retinas (Ts'o and Friedman, 1967; Hogan et al., 1971). Their presence in the irradiated retina may not have been resulted from irradiation. However, cytoplasmic organelles in pigment epithelial cells were altered severely (Fig. 5) although these cells receive their nutrients from choroidal blood vessels, which showed no morphological evidence of irradiation damage. In the foveal sample pigment epithelial cells lost most of their pigment and separated from Bruch's membrane. Thus, it is possible that the pigment epithelial cells were damaged directly by irradiation.

Henkind and Gartner (1983) and Korte et al. (1984) discovered that damage to the pigment epithelium leads to atrophy of the choroidocapillaris. Survival of photoreceptor cells also depends on the pigment epithelium. Thus, if radiation damaged foveolar pigment epithelial cells particularly severely, the disappearance of the choroidocapillaris (Henkind and Gartner, 1983; Korte et al., 1984) and of the cone cells from the fovea is explained. This foveal injury led to the loss of Henle's fiber layer in the parafoveal region of the treated retina (Fig. 2).

Although not seen any more in the excised eye, telangiectatic vessels were detected below the optic disc before the radiation cataract prevented inspection of the retina in 1978. Thus, a macular edema due to venous occlusion may have developed. The foveal cysts (Fig. 3) may have resulted from the edema.

Ultrastructural studies on human retinas with radiation retinopathy are not available. Irvine et al. (1981) and Irvine and Wood (1987) have produced radiation retinopathy in capuchin monkeys. They found intraretinal vessels with thick collagenous walls, similar to those seen in this case (Figs. 2, 9). They also described large cysts within the retinal tissue which resemble those shown in Fig. 3.

The spatial density of bipolar cells in the periphery and parafovea of the human retina is not known. In the retina of cynomolgus monkeys at 30° eccentricity, Krebs and Krebs (1987) have found 50,000 bipolar cells per mm<sup>2</sup> retinal area. The density of photoreceptor cells at this location is higher in the macaque (8,200 cones and 160,000 rods/mm<sup>2</sup>: Krebs and Krebs, 1987, 1991) than in the human (6,000 cones and 120,000 rods/mm<sup>2</sup>: Osterberg, 1935). From this it follows that the density of bipolar cells in the normal human retina at 30° eccentricity may be significantly below 50,000 cells/mm<sup>2</sup>. Thus, the value of 34,000 bipolar cells/mm<sup>2</sup> in the periphery of the irradiated retina (Table 1) may indicate only a small loss of bipolar cells. More bipolar cells appear to have been lost in the parafovea, where a greater density of bipolar cells might be expected due to higher cone cell density. The relatively greater loss of bipolar cells in the parafovea (Table 1) may have resulted from the loss of foveal cone cells.

Radiation also may produce atrophy of the optic nerve (Wara et al., 1979; Riedel et al., 1983). The number of axons in the human optic nerve is given as about 1,2 million by Hogan et al. (1971) and by Balazsi et al. (1984). However, the number of fibers may decrease with age (Balazsi et al., 1984; Johnson et al., 1987; Repka and Quigley, 1989). Our fiber count in the irradiated optic nerve is slightly lower than the lowest value given by Balazsi et al. (1984) for corresponding ages (1.1 million). The number of one million nerve fibers in the irradiated eye indicates that a reduction of the ganglion cell number may have occurred; but this reduction may not have been substantial.

Fiber diameter in the human optic nerve decreases with age (Balazsi et al., 1984). A higher value is to be expected in a 17 year old than in a 58 year old person. The relative axon area (Table 2) is directly proportional to the mean fiber diameter. We found a lower value (Table 2) in the nerve of the younger, irradiated patient. The difference in the thickness of the myelin sheath of the axons is even more pronounced (Table 2). Decrease in fiber diameter and thinning of the myelin sheath in the irradiated eye (Table 2) may be due to radiation injury. Chemotherapy may have contributed. However, Hogan et al. (1971) stated the range of the thickness of the myelin sheath as being between 30 and 270 nm in normal human optic nerves. The thickness of the myelin sheath in the non-irradiated nerve (Table 2) exceeds Hogan et al. range. Further study of the normal optic nerve is needed to clarify this discrepancy.

In conclusion, irradiation, possibly enhanced by chemotherapy (Chan and Shukovsky, 1976), may have led to degenerative changes in the retinal blood vessels and/or in the pigment epithelium. A secondary slow loss of photoreceptor cells may have followed, rod cells being more affected than cone cells. Rod and cone outer segments vanished almost completely. A reduction in the number of higher order neurons, such as bipolar and ganglion cells, may have resulted from the loss of stimulation by photoreceptor cells.

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