APOPTOSIS IN HUMAN RETINAL DEGENERATIONS*

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ABSTRACT

Purpose: This paper examined the role of apoptosis in human retinal degenerations including pathologic myopia, age-related macular degeneration, serous retinal detachment, retinal lattice, and paving stone degenerations.

Method: Thirty-seven enucleated human eyes with 1 of the above-mentioned retinal degenerations were studied by histopathology and by TdT-mediated biotin-dUTP nicked-end labelling (TUNEL) technique.

Results: Tunnel labelling characteristic DNA fragmentation of apoptosis was observed in photoreceptor cells in 2 of the 4 eyes with pathologic myopia and in 4 of 16 eyes with age-related macular degeneration, 2 of which were exudative and 2 of which were atrophic. However, only a few scattered photoreceptor cells were labelled in 4 of 8 eyes with serous retinal detachment secondary to malignant melanoma of the choroid. Moreover, none of the photoreceptors cells in the 4 eyes with retinal lattice degeneration and 6 eyes with retinal paving stone degeneration were labelled.

Conclusions: Apoptosis is 1 of the important pathways of photoreceptor cell degeneration in pathologic myopia and age-related macular degeneration.

INTRODUCTION

Photoreceptor cell death is a hallmark of a number of macular degenerations, such as age-related macular degeneration, myopic macular degeneration, photic maculopathy, traumatic maculopathy, cone-rod dystrophy and others.[1-3] It is also found in some forms of peripheral retinal degener-

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ations, such as paving stone degenerations and lattice degeneration. However, the mechanisms of photoreceptor cell death have not been determined. Understanding the mechanisms of the photoreceptor cell death should lead to additional avenues for photoreceptor rescue.

Apoptosis, also termed program cell death, is a specific form of cell demise initiated by an endogenous cellular process.[4] Apoptosis (from the Greek word *falling off*) is widely observed in the biological world, ranging from the falling of leaves in the autumn, to metamorphosis of the caterpillar to a butterfly, to regression of the lactating mammary glands. This type of programmed cell death is believe to be triggered by a gene or sets of genes and is commonly seen in embryologic, physiologic, or pathologic processes. In embryogenesis, the size and shape of organs are regulated by selective deletion of cells by apoptosis. In physiologic programs, apoptosis leads to hormone-mediated regression of uterine epithelium in menstruation, regression of the lactating mammary gland, and deletion of certain lymphocytic cones. In pathologic conditions, apoptosis is seen in radiation injury, tumor regression in retinoblastoma, aging conditions such as Alzheimer's disease, and hereditary degenerations such as retinitis pigmentosa.

This process is characterized biochemically by double-stranded DNA degradation in which the genome is cleaved at the internucleosomal sites, producing fragments of multiples of 180 to 200 base pairs, which may be demonstrated by agarose gel electrophoresis as a ladder pattern. The endonuclease, which brings about the DNA degradation, is calcium- and magnesium-dependent. This process appears to require *de novo* protein synthesis and may be triggered by a rise in intracellular calcium level. Morphologically, apoptotic cells characteristically exhibit condensation of chromatin at the nuclear periphery, nuclear disintegration, reduction in nuclear size with formation of apoptotic bodies, and progressive degeneration of the residual nuclear and cytoplasmic structures.

This form of cell death has recently been recognized in conventional histologic sections of various organs by Gavrieli and associates[5] by an *in situ* method of visualization of nuclear DNA fragmentation. This DNA nicked-end labelling with biotinylated dUTP after incubation with a terminal deoxynucleotidyl transferase (TUNEL) is useful to identify fragmented DNA and apoptosis. TUNEL labelling detects DNA fragmentation at the single cell level.

In recent ophthalmic literature, apoptosis of photoreceptor cells has been described in embryologic development of human retina,[6] photic retinopathy,[7] retinoblastoma,[8] retinitis pigmentosa,[9] hereditary retinal dystrophy of RCS rats,[10] retinal degeneration of rd and rds mice,[11] and trans-

genic mice expressing a rhodopsin mutation that causes retinitis pigmentosa in humans and others.[12] In a more recent article, degeneration of photoreceptor cells, especially the cone cells, was observed in human traumatic detached retina.[13]

This article presents further evidence of apoptosis cell death in the human photoreceptor degeneration in myopic macular degeneration and age-related macular degeneration and in serous detachment of retina secondary to choroidal malignant melanoma. It appears that apoptotic cell death is a common form of photoreceptor cell death in many retinal degenerations.

METHODS

CASE SELECTION

Thirty-seven enucleated human eyes, 30 from the autopsy of Chinese patients at the Prince of Wales Hospital in Hong Kong and 7 from surgical enucleation from the Georgiana Theobald ophthalmic pathology laboratory, UIC Eye Center, Chicago, were examined in this study. Four eyes exhibited pathologic myopia with axial length greater than 28 mm. Sixteen eyes had age-related macular degeneration (ARMD). Four eyes showed peripheral retinal lattice degeneration. Six had peripheral retinal paving stone degeneration. Seven eyes exhibited serous retinal detachment secondary to choroidal malignant melanoma.

TISSUE PREPARATION

All eyes were cut in horizontal strips (6 x 9 mm) with optic discs and maculas fixed in 10% buffered formaldehyde, dehydrated, and embedded in paraffin. Serial paraffin sections 6 μm thick were cut. The sections were stained with hematoxylin-eosin and were examined by light microscopy.

TDT-MEDIATED BIOTIN-DUTP NICK-END LABELLING (TUNEL)

We studied these human eyes by TdT-mediated biotin-dUTP nicked-end labelling (TUNEL) technique. Apoptosis Detection Kit (S7100-Kit, Oncor Co, Inc) was used for DNA nicked-end labelling. For positive control, sections from the retina of a 35-day-old RCS rat were used in each group (Fig 1). In addition, 1 normal retina from a 65-year-old human (the time interval between death and eyeball fixation was 72 hours) was used for TUNEL labelling to determine if postmortem body storage time might have an effect in TUNEL labelling. As a negative control, adjacent serial sections of retinas were processed for TUNEL labelling following the standard procedure but omitting incubation with TdT or biotinylated dUTP in TdT buffer during DNA nicked-end labelling.

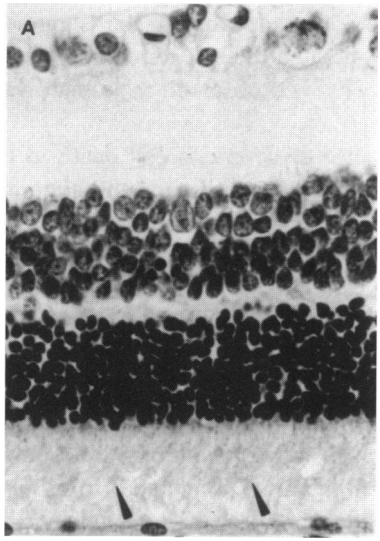

FIGURE 1A

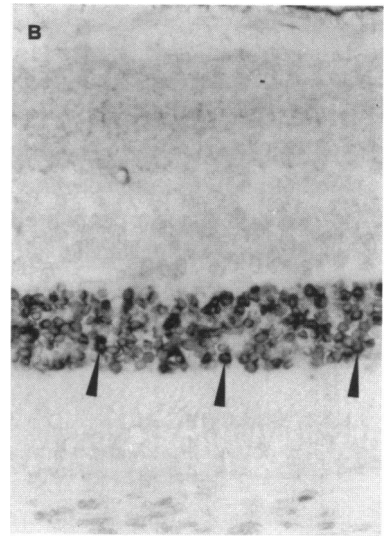

FIGURE 1B

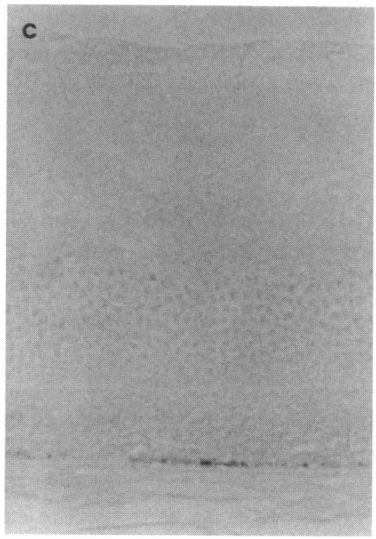

FIGURE 1C

FIGURE 1

Retina of RCS rat (positive control and negative control). A. Retina of a 35-day-old RCS rat, the outer and inner segments were grossly misaligned (arrowheads) hematoxylin-eosin, x400). B. Great majority of the photoreceptor nuclei were labelled by TUNEL technique. Most of the nuclei were labelled with a ring configuration (arrowheads) (x400). C. Negative control for TUNEL technique (x400).

RESULTS

PATHOLOGIC MYOPIA

In 4 autopsy eyes, a diagnosis of pathologic myopia was made by gross and

histopathologic features. Grossly, these eyes showed increased axial length, which ranged from 28 to 31mm with posterior staphyloma and scleral thinning. A tilted optic disc, myopic crescent, and liquifaction and posterior detachment of the vitreous were observed in all 4 eyes. The peripheral retina in these eyes displayed extensive pigmentary degeneration. Paving stone retinal degeneration was observed in 1 eye. Histopathologically, the retina was markedly thinned in all 4 eyes, especially in the posterior pole. Schisis of retinal nerve fiber layer was noted in the equatorial area in 2 eyes. The inner nuclear layer was unremarkable in 4 eyes. Reduction of the outer nuclear layer (ONL) was noted in all 4 eyes, especially at the posterior staphylomatous area. In focal areas of the macula, the ONL was reduced to 1 to 2 nuclei thick (Fig 2). There was shortening of the outer segments and inner segments or focal total loss of these photoreceptor elements (Figs 3 and 4). Some photoreceptor cells were dislodged into the subretinal space. The peripheral retina showed cystoid degeneration in all 4 eyes. The RPE cells were remarkably atrophic or absent focally in the posterior pole. The markedly atrophic choroid exhibited attenuation and absence of the choriocapillaris at the posterior pole. The scleral thickness was markedly reduced in these eyes, especially at the posterior pole.

In the TUNEL labelling study, 2 of 4 eyes with pathologic myopia showed positively labelled photoreceptor nuclei, some of which were labelled homogeneously and others with a ring configuration. The scat-

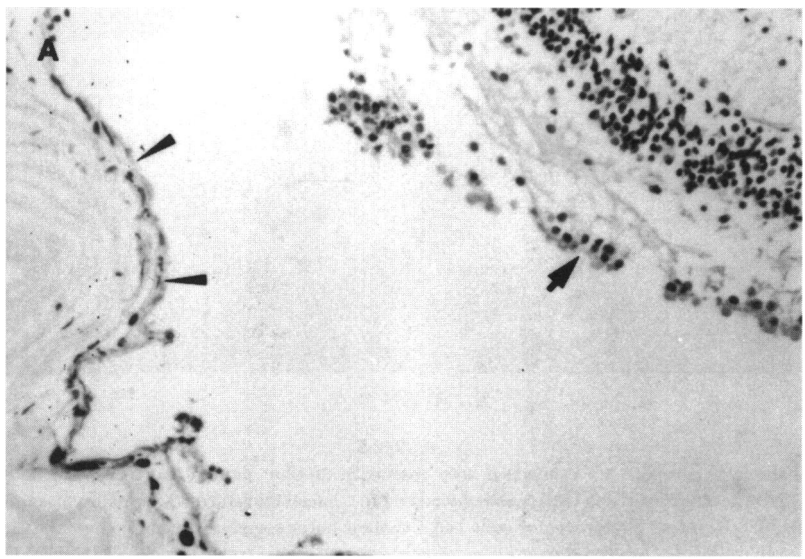

FIGURE 2A

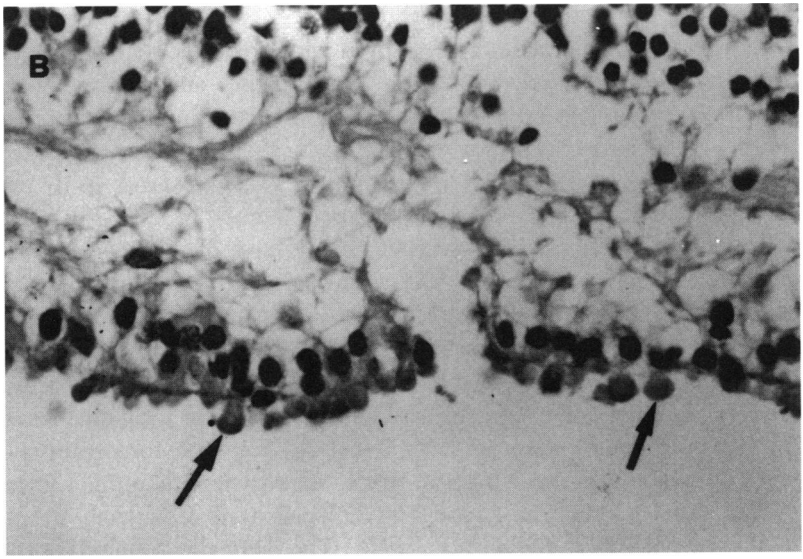

FIGURE 2B

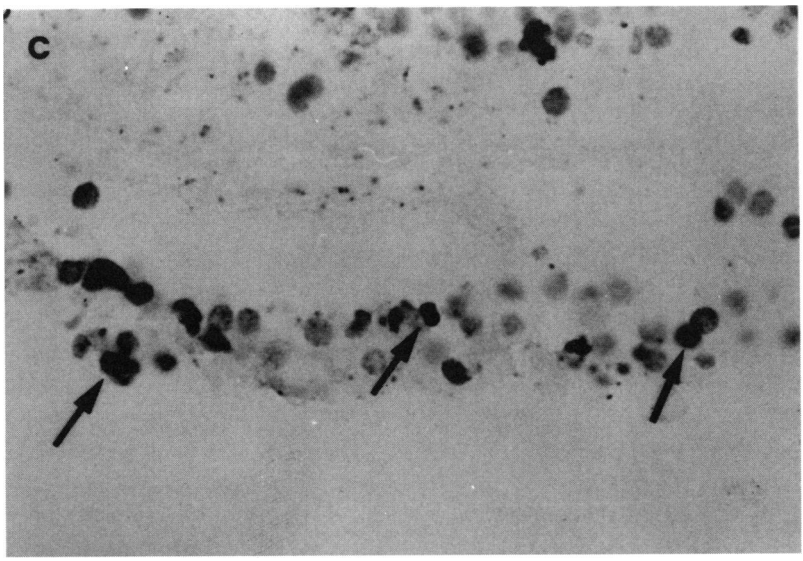

FIGURE 2C

FIGURE 2

Pathologic myopia. A. In macular area, markedly atrophic choroid and RPE were noted (arrowheads). Overlying ONL was reduced to 1 to 2 nuclei thick (arrow) (hematoxylin-eosin, x100). B. Most photoreceptor cells had lost their outer segments (arrows) (hematoxylin-eosin, x400). C. Scattered photoreceptor cells were labelled (arrows) by TUNEL technique showing evidence of apoptosis (hematoxylin-eosin, x400).

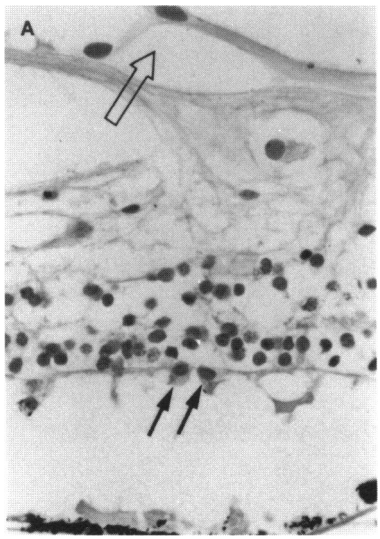

FIGURE 3A

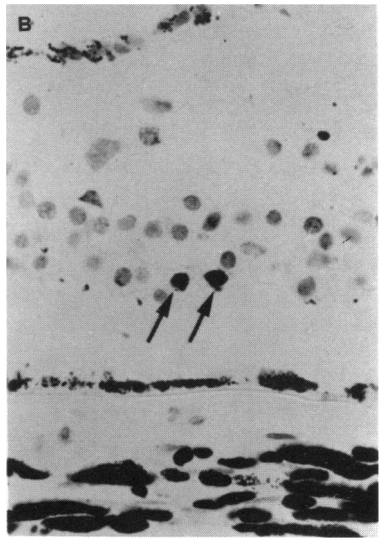

FIGURE 3B

FIGURE 3

Pathologic Myopia A. At equatorial area, note absence of choriocapillaris and markedly atrophic RPE. A few remaining photoreceptor nuclei extruded from outer limiting membrane into subretinal space (arrows) (hematoxylin-eosin, x400). B) Photoreceptor nuclei, extruded from outer limintting membrane, were labelled (arrows) by TUNEL technique (x400).

tered labelled photoreceptor cells were more prominent at the macular area than those at the equatorial and peripheral retina. Loss of the photoreceptor elements was a common feature in the labelled photoreceptor cells. Some dislodged photoreceptor cells in the subretinal space were also labelled. The TUNEL- positive photoreceptor nuclei were usually observed in areas where marked reduction of the thickness in the ONL was noted. In addition, a few cells in the inner nuclear layer were also labelled in 1 eye.

Two of the 4 eyes with pathologic myopia showed no TUNEL labelling in any of their retinal cells. One of them had a cataract extraction operation before death.

AGE-RELATED MACULAR DEGENERATION

Sixteen eyes were diagnosed with ARMD by histopathologic criteria. All eyes had normal inner limiting membrane, nerve fiber layer, ganglion cell layer, and INL, and pathologic findings were limited to the outer retinal layers. There was mild to severe reduction of ONL thickness in the macular area in 12 eyes. In each of these 12 eyes, atrophic RPE, drusen (Figs

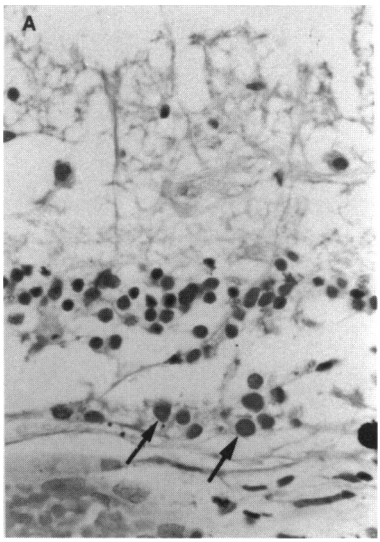

FIGURE 4A

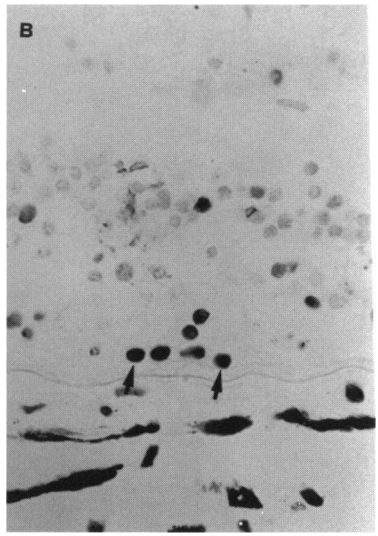

FIGURE 4B

FIGURE 4

Pathologic myopia. A. Temporal to macular area, some photoreceptor cells were devoid of outer and inner segments and lined Bruch's membrane (arrows) (hematoxylin-eosin, x400). B. These photoreceptor nuclei were labelled (arrows) by TUNEL technique (x400).

5 and 6), and irregularly thickened Bruch's membrane consistent with the atrophic form of ARMD were observed. In each of the 4 eyes, a subretinal or sub-RPE neovascular membrane was noted (Fig 7). The ONL of these eyes was reduced to 1 or 2 nuclei thick or totally disappeared focally. Shortening of the outer and the inner segments of photoreceptor cells or total loss of these structures was common. Some photoreceptor cells were dislocated into the subretinal space. Fragmentation and margination of the chromatin in photoreceptor cells was also noted occasionally.

In the TUNEL labelling study, 4 of 16 ARMD eyes showed positively labelled photoreceptor cells. Two of these 4 exhibited the exudative form of ARMD and the other 2 had the atrophic form of ARMD. The labelled photoreceptor cells showed a scattered distribution. Most were labelled homogeneously, and a few were labelled with a ring configuration. Some labelled photoreceptor cells were found in the subretinal space. Loss of the photoreceptor elements was a characteristic of these labelled photoreceptor cells. The labelled photoreceptor nuclei were usually observed in the areas where marked reduction of thickness in the ONL was seen. In addition to the labelled photoreceptor cells, a few cells in the INL were also labelled in 1 eye.

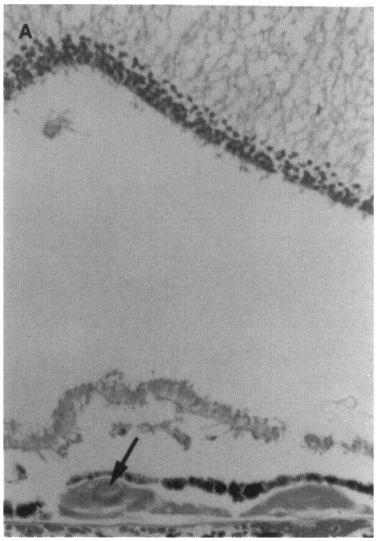

FIGURE 5A

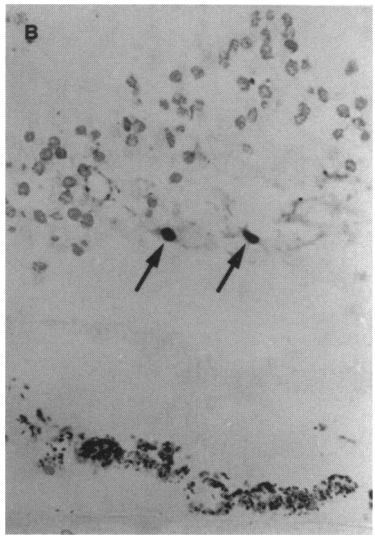

FIGURE 5B

FIGURE 5
Drusen of retinal pigment epithelium. A. Large drusen (arrow) overlaid by thinned RPE, and moderate thinning of outer nuclei layer were seen (hematoxylin-eosin, x200). B. Scattered photoreceptor nuclei were labelled (arrows) by TUNEL technique (x400).

SEROUS RETINAL DETACHMENT

Clinical diagnosis of serous retinal detachment secondary to choroidal malignant melanoma was made in 7 eyes and confirmed histopathologically. All of the choroidal malignant melanomas were of the mixed cell type. The melanomas were observed in the posterior pole in 6 eyes, and 1 tumor arose from the ciliary body and anterior peripheral choroid. The size of the tumors varied from 7 x 7 x 6 mm to 16 x 14 x 11 mm, and all the malignant melanomas invaded the Bruch's membrane and extended into the subretinal space and the overlying retina. The tumor extended into the vitreous cavity in 2 cases and into the optic nerve head in 3 cases. The optic nerve heads were atrophic and gliotic in 2 eyes. In 2 other eyes, the optic nerve heads were hemorrhagic and swollen. In 4 of 7 eyes, vitreous hemorrhage was observed.

The RPE overlying the tumors showed marked atrophic degeneration or reactive proliferation. Drusenoid deposits on the Bruch's membrane were noted in 6 eyes. In all 7 cases, the retina overlying the tumor showed cystoid degeneration. The architecture of the retina was markedly disrupted in 3 eyes. Extensive serous retinal detachment with pigment-laden macrophages scattered throughout the subretinal space was found in all

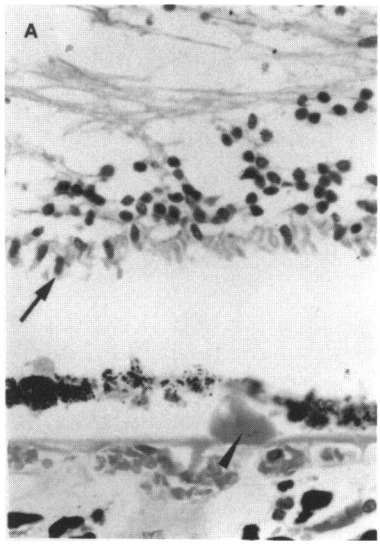

FIGURE 6A

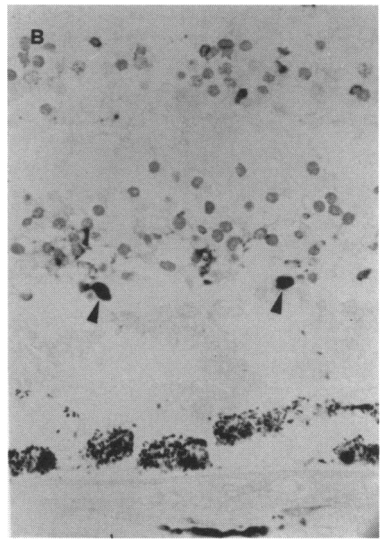

FIGURE 6B

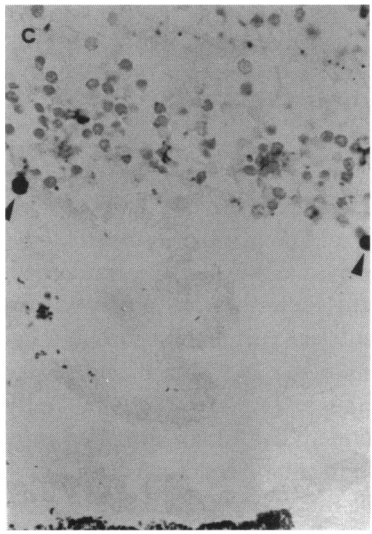

FIGURE 6C

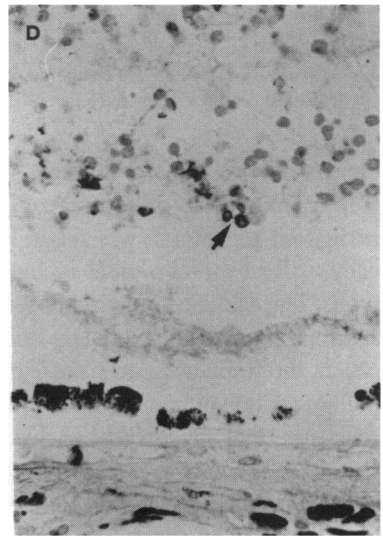

FIGURE 6D

FIGURE 6

Atrophic macular degeneration. A. Macular area showing moderate thinning of overlying outer nuclear layer. One druse (arrowhead) was noted beneath thinned RPE. Some photoreceptor nuclei were dislocated into inner segments (arrows) (hematoxylin-eosin, x400). B, C, and D. Scattered photoreceptor nuclei were labelled intensely homogeneously (arrowheads) or with a ring configuration (arrow) by TUNNEL technique (x400).

FIGURE 7A

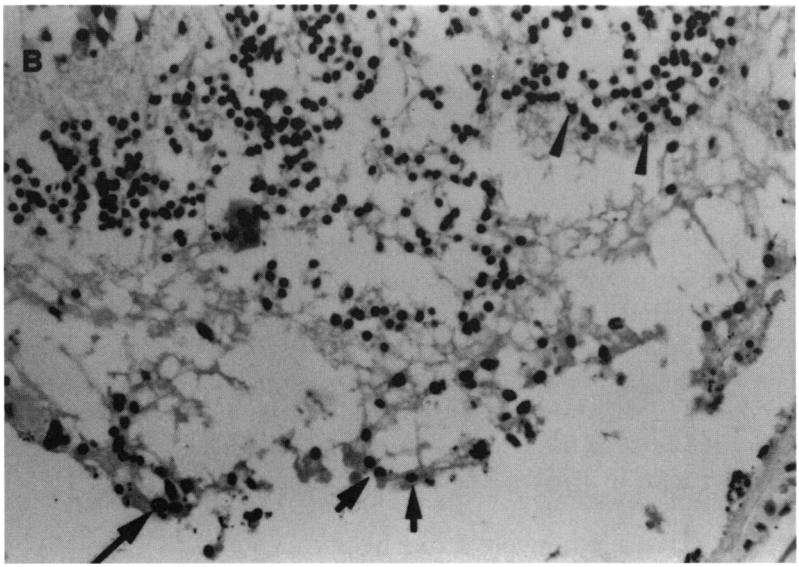

FIGURE 7B

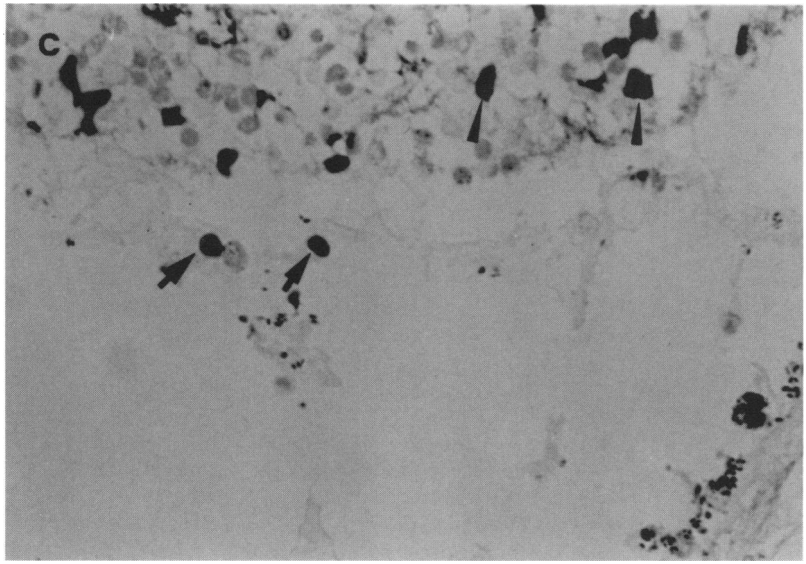

FIGURE 7C

FIGURE 7

Age-related exudative macular degeneration. A. A distinct fibrovascular membrane was noted in subretinal space (between long arrows) (hematoxylin-eosin, x100). B. Photoreceptor cells in marked atrophic outer nuclear layer, which had only one or two rows of remaining photoreceptor cells, exhibited loss of outer and/or inner segments (short arrows). Margination of chromatin in photoreceptor nuclei was noted (long arrow) (hematoxylin-eosin, x200). C. Scattered photoreceptor nuclei were labelled (arrows) and some cells of INL were also labelled (arrowheads) by TUNEL technique (x400).

eyes (Fig 8). Extensive or focal loss of photoreceptor cells in the serously detached retinas was seen in all retinas.

In the TUNEL study, few scattered photoreceptor cells were labelled in 4 eyes. Some labelled photoreceptor cells were dislocated into the subretinal space and showed loss of the photoreceptor elements. Labelled photoreceptor cells were usually observed in areas where marked reduction of thickness of the ONL was seen. Scattered labelled tumor cells were also noted in 2 of the 7 choroidal malignant melanomas.

RETINAL LATTICE AND PAVING STONE DEGENERATIONS

Peripheral retinal lattice degeneration was observed in 4 eyes that were otherwise normal. Grossly, the lesions appeared as circumscribed atrophic areas with sharp edges, located between the equator and the ora serrata. A few whitish blood vessels crossed the lesional areas, where pigment

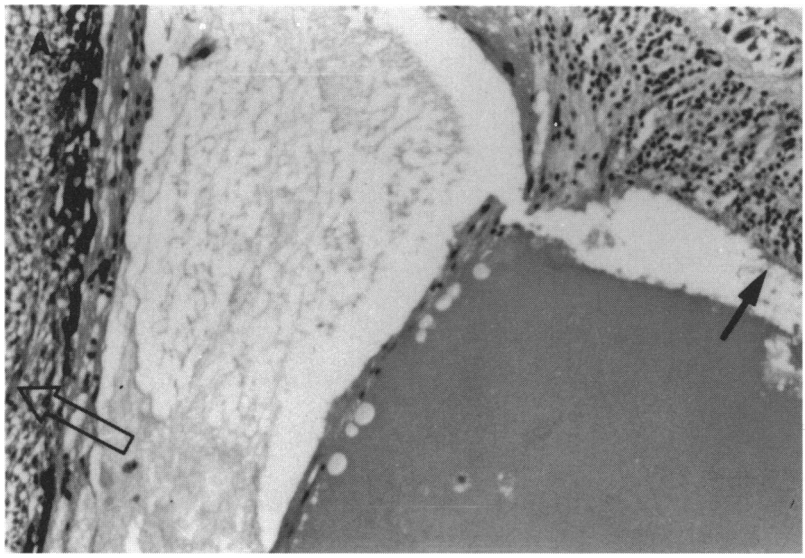

FIGURE 8A

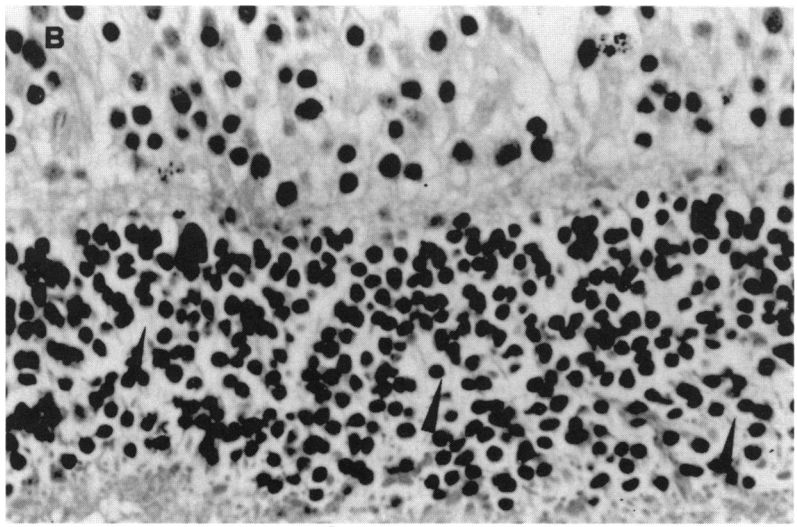

FIGURE 8B

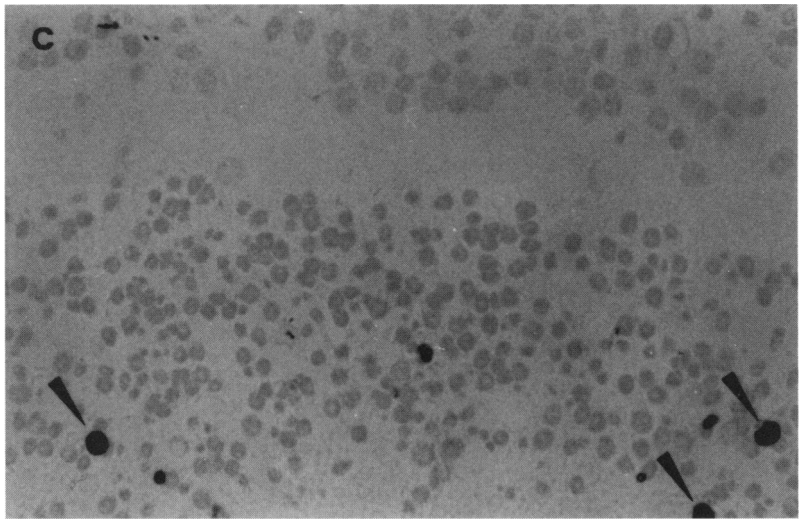

FIGURE 8C

FIGURE 8

Serous retinal detachment secondary to malignant melanoma of choroid. A. Serous retinal detachment (arrow) secondary to malignant melanoma of choroid (hollow arrow, mixed cell type) (hematoxylin-eosin, x100). B. In detached retina, edema of ONL was noted (arrowheads) (hematoxylin-eosin x400). C. Scattered photoreceptor nuclei were labelled with a ring configuration (arrowheads) by TUNEL technique (x400).

deposition was also observed. Histopathologically, the retinas were moderately thinned and showed atrophy and degeneration of all retinal layers, especially the inner nuclear layer (Fig 9). Thickened and sclerotic retinal blood vessels were focally surrounded by proliferated pigment epithelial cells. The overlying vitreous was liquefied with condensation and adherence of vitreous strands at the edges of the lesions. In some eyes, a glial membrane overlying the anterior surface of the atrophic retina was also seen.

Retinal paving stone degeneration was observed in 6 eyes that were otherwise normal. Grossly, these peripheral retinal lesions were ovoid in shape, occurring singly or in groups. They were located posterior to the ora serrata. Prominent choroidal vessels were observed at the base of the lesions. Microscopic examination of the lesions revealed thinning of the retina, especially the outer nuclear layer with distinct atrophy of RPE and absence of choriocapillaris (Fig 10). The atrophic retina was adherent to the Bruch's membrane. No inflammatory response was found in the retina.

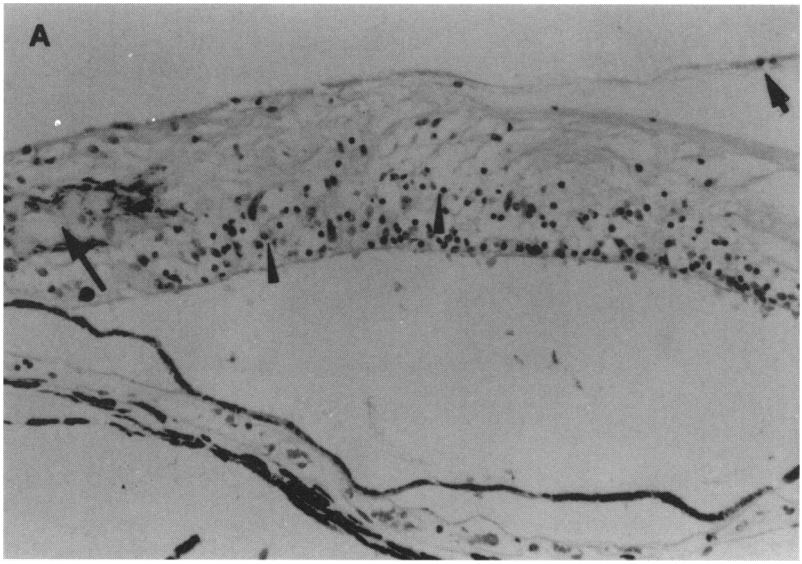

FIGURE 9A

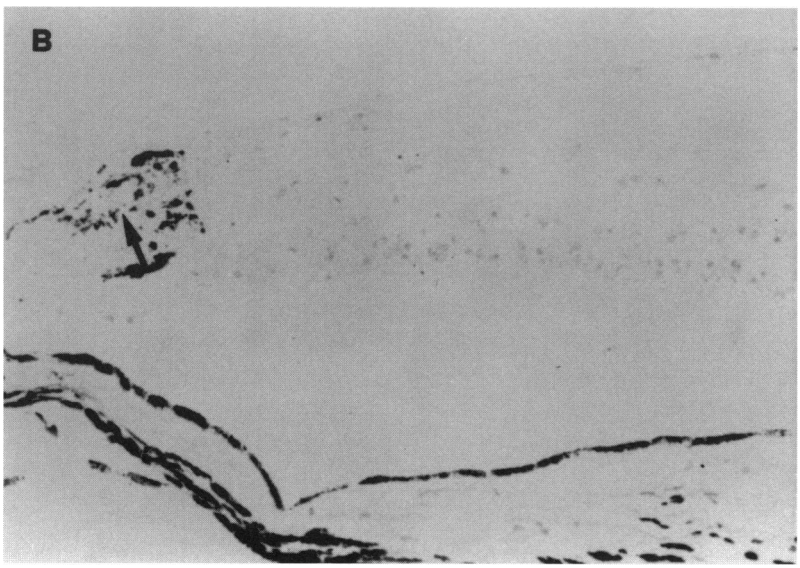

FIGURE 9B

FIGURE 9

Peripheral retinal lattice degeneration. A. In degenerated area, disorganized retina with marked thinning of inner and outer nuclear layers was seen (arrowheads). A sclerotic and occluded retinal artery within lesion was surrounded by proliferated RPE cells (long arrow). A preretinal glial membrane overlying the lesion was noted (short arrow) (hematoxylin-eosin, x200). B. No retinal cells were labelled by TUNEL technique (x200).

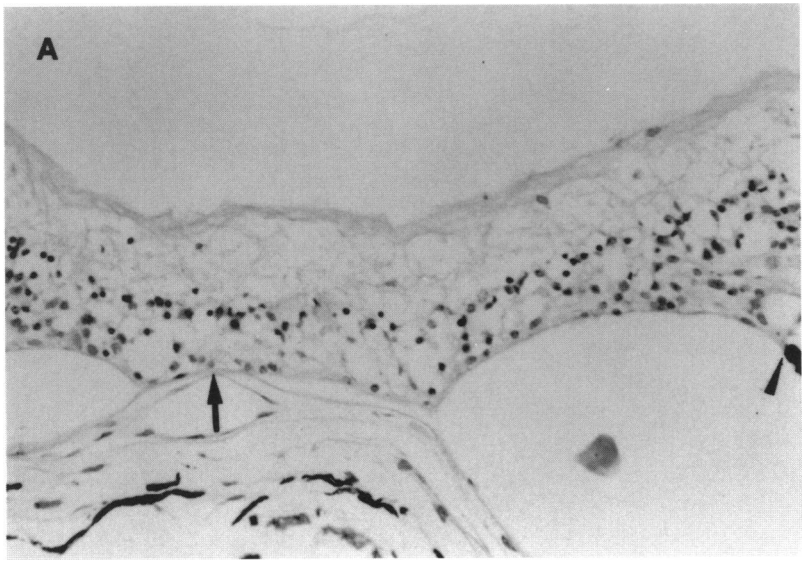

FIGURE 10A

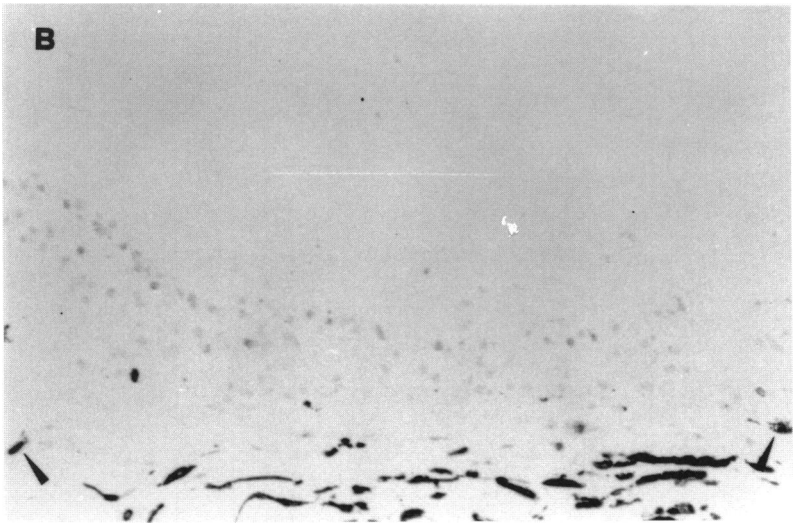

FIGURE 10B

FIGURE 10

Paving stone degeneration in peripheral retina. A. RPE ended (arrowheads) abruptly in area of degeneration, where there was absence of choriocapillaris and retinal pigment epithelium (arrow). Overlying retina exhibit extensive atrophy, especially in outer nuclear layer (hematoxylin-eosin, x200). B. None of retinal cells was labelled by TUNEL technique (x200).

In the TUNEL labelling study, eyes with peripheral retinal lattice degeneration and paving stone degeneration failed to show any positively labelled retinal cells.

DISCUSSION

In this study, we examined 37 human eyes with various types of retinal degenerations with TUNEL labelling technique for evidence of apoptosis. Labelled photoreceptor cells were noted in 2 of the 4 eyes with pathologic myopia, and 4 of the 16 eyes with ARMD. Gavrieli and coworkers showed that the TUNEL technique labels the 3' end of the DNA fragments of the apoptotic nuclei, and internucleosomal DNA fragmentation is a hallmark of apoptosis. Therefore, we conclude that apoptosis is 1 of the important mechanisms of photoreceptor cell death in pathologic myopia and ARMD.

In 4 of 7 eyes with serous retinal detachment secondary to malignant melanoma of choroid, few photoreceptor nuclei were labelled. In contrast, traumatic retinal detachment in humans had many labelled photoreceptors cells. Apoptosis might not be a prominent mechanism in this secondary serous retinal detachment.

TUNEL technique failed to demonstrate positive labelling of retinal cells in 6 eyes with paving stone degeneration. Choroidal vascular insufficiency is believed to be responsible for the development of paving stone degeneration.[14] Curtin[15] reported increased prevalence in patients over age 40 and in eyes with long axial length. Similar retinal lesions had also been reported in patients with unilateral hypotensive retinopathy.[16] Because ischemia is reported to induce apoptosis in neurons, we speculated that the apoptotic process might be involved in the retinal cell death in retinal paving stone degeneration, but we failed to demonstrate labelled cells. However, we may not have studied these lesions in the active degenerative phase.

The TUNEL technique also failed to show positive labelling in photoreceptor cells in eyes with lattice retinal degeneration. The pathogenesis of lattice degeneration remains unknown. Hypotheses of the pathogenesis of lattice degeneration include (1) vitreous traction,[17] (2) retina ischemia,[18] and (3) a primary defect in the internal limiting membrane of the retina.[19] Indeed, breaks in this membrane might stimulate proliferation of preretinal glial membranes overlying the retinal lesions.[18] Growth factors such as bFG induced preretinal glial membrane proliferation and might inhibit the apoptotic process.

These chronic degenerations of the photoreceptor cells are a lifelong process, but the short-lived apoptotic process, which is usually completed

in 24 hours, might only be detected in certain periods of these degenerative diseases. In addition, there might be certain periods when the apoptotic process was active. For example, in experimental traumatic retinal detachment, the first apoptotic peak is detected on the third day after detachment, and the second apoptotic peak is detected around day 14 after retinal detachment.[20] The apoptotic process might appear in several periods of the chronic retinal degenerative process. This might be one of the reasons why apoptosis was not observed in the peripheral paving stone or lattice degenerations.

In the eyes with pathologic myopia, ARMD and serous retinal degeneration, there was absence of inflammatory response and abundant cellular debris in the retinas, even though the eyes were in advanced-stage disease. This observation was consistent with cell death by apoptosis, an energy-requiring process of self-elimination of cells without production of cellular debris that attract inflammatory cells. In sharp contrast to cell death by necrosis, inflammatory reaction is inevitably seen.[21,22]

Two of the 4 eyes of pathologic myopia failed to show positive labelling with TUNEL technique. One of the patients underwent cataract extraction 1 year ago and had cystoid macular edema after surgery. Recent studies showed a high level of basic fibroblast growth factor (bFGF) in human and animal aqueous humor after cataract surgery.[23] bFGF was believed to be one of the inhibitors of apoptosis[24,25] and had been demonstrated to delay photoreceptor degeneration in retinal dystrophy in rats.[26] bFGF might have inhibited the apoptotic process of photoreceptor cells in this patient.

In this study, TUNEL-labelled apoptotic photoreceptor cells were observed in 2 of 4 eyes with exudative ARMD and subretinal neovascular membrane. On the other hand, 2 of 12 eyes with atrophic ARMD without subretinal neovascular membranes had apoptotic photoreceptor cells. Subretinal neovascular membrane might be a strong stimulus that triggered apoptosis of photoreceptor cells. The endothelial cells in the neovascular network lacked blood-retinal barrier and leaked lipoproteinaceous fluid into the sub-RPE or subretinal space. In addition, these fragile new vessels were prone to hemorrhage. Lipid peroxidation, which resulted from exudation and hemorrhage, might trigger apoptosis of photoreceptor cells in ARMD with subretinal neovascular membranes.

Recently, Tso and associates[10] reported that apoptosis was a primary mechanism of photoreceptor cell degeneration in inherited retinal dystrophy in the RCS rat. Studies of mouse models of retinitis pigmentosa also showed that apoptosis was a final common pathway of photoreceptor cell death.[11] In other studies of acquired retinal degeneration, apoptotic pho-

toreceptor cells were observed in experimental retinal detachment [27,28] and experimental photic retinopathy.[7,29,30] Furthermore, Chang and associates also demonstrated that apoptosis appears to be a primary mechanism of human photoreceptor cell death following traumatic retinal detachment. This study additionally demonstrates apoptosis in human myopic macular degeneration and age-related macular degeneration.

REFERENCES

1. Alan CB.Retinal photoreceptor dystrophies. *Am J Ophthalmol* 1995; 119:543-562.
2. Tso MO. Pathogenetic factors of aging macular degeneration. *Ophthalmology* 1985; 92:628-635.
3. Tso MO. *Retina Disease: Biomedical Foundations in Clinical Management.* Philadelphia, Lippincott,1988.
4. Tomei LD, Cope FO. *Apoptosis : The Molecular Basis of Cell Death.* New York,Clo Spring Harbor Lab Press, 1991.
5. Gavrieli Y, Sherman Y, Ben-Sasson. Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. *J Cell Biol* 1992; 119:493-501.
6. Gregory CY, Bird AC. Cell loss in retinal dystrophies by apoptosis death by informed consent. *Br J Ophthalmo.* 1994; 232:635-645.
7. Abler AS, Chang CJ, Fu J, et al. Photic injury triggers apoptosis of photoreceptor cells. (ARVO abstract) *Invest Ophthalmol Vis Sci* 1994; 35:S1517.
8. Buchi ER, Bernauer W, Daicker B. Cell death and disposal in retinoblastoma: an electron microscopic study. *Graefes Arch Clin Exp Ophthalmol* 1994; 232:635-645.
9. Portera-Cailliau C, Sung CH, Nathans T, et al. Apoptotic cell death in mouse models of retinitis pigmentosa.*Proc Natl Acad Sci U S A* 1994; 91:974-978.
10. Tso MO,Zhang C, Abler AS, et al. Apoptosis leads to photoreceptor degeneration in inherited retinal dystrophy of RCS rats. *Invest Ophthalmol Vis Sci* 1994; 35:2693-2699.
11. Chang GO, Hao Y, Wong F. Apoptosis: final common pathway of photoreceptor death in rd, rds, and rhodopsin mutant mice. *Neurone* 1993; 11:595-605.
12. Szczesny PJ. The early stages of photoreceptor degeneration in various forms of retinitis pigmentosa in man suggest apoptosis as a common pathway (ARVO abstract) *Invest Ophthalmol Vis Sci* 1995; 36:S919.
13. Chang CJ, Lai WW, Tso MO. Apoptotic photoreceptor cell death after traumatic retinal detachment in humans. *Arch Ophthalmol* 1995; 113:880-886.
14. Karlin DB, Curtin BJ. Peripheral chorioretinal lesions and axial length of the myopic eye. *Am J Ophthalmol* 1976; 81:625-635.
15. Curtin BJ. *The Myopias: Basic Science and Clinical Management.* Philadelphia, Harper & Row, 1985.
16. Michelson PE, Knox DL, Green WR. Ischemic ocular inflammation: a clinicopathologic case report. *Arch Ophthalmol* 1971; 86:274-280.
17. Foos RY, Simons KB. Vitreous in lattice degeneration of retina. *Ophthalmology* 1984; 91:452-457.
18. Robinson MR, Steeten BW. The surface morphology of retinal break and lattice retinal degeneration: a scanning electron microscopic study. *Ophthalmology* 1986; 93:237-246.
19. Streeten BW, Bert M. The retinal surface in lattice degeneration of the retina. *Am J Ophthalmol* 1972; 74:1201-1209.
20. Cook B, Lewis GP, Fisher SK, et al. Apoptotic photoreceptor degeneration in experimental retinal detachment. *Invest Ophthamol Vis Sci.* 1995; 36:990-996.
21. Wyllie AH. Glucocorticoid induced thymocytes apoptosis is associated with endogenous endonuclease activation. *Nature.* 1980; 284:555-556.

22. Kerr JF, Harmon BV. *Apoptosis: The Molecular Biology of Cell Death.* New York, Cold Spring Harbor Press, 1991, pp 5-30.
23. Namiki M. Quantification of basic fibroblast growth factor (bFGF) and transforming growth factor (TGF alpha) in rabbit aqueous humor after intraocular lens implantation. *Nippon Ganka Gakkai Zasshi* 1994; 98:334-339.
24. Fuks Z, Persaud RS, Alfieri A, et al. Basic fibroblast growth factor protects endothelial cells against radiation-induced programmed cell death in vitro and in vivo. *Cancer Res* 1994; 54:2582-2590.
25. Haimovitz-Friedman A, Balaban N, Mcloughlin M, et al. Protein kinase C mediates basic fibroblast growth factor protection of endothelial cells against radiation-induced apoptosis. *Cancer Res* 1994; 54:2591-2597.
26. Faktorovich EG, Steinberg RH, Yasumura D, et al. Photoreceptor degeneration in inherited retinal dystrophy delayed by basic fibroblast growth factor. *Nature*.1990; 347:83-86.
27. Cook B, Lewis GP, Adler R, et al. Photoreceptor degeneration in experimental retinal detachment: evidence for an apoptotic mechanism. (ARVO abstract) *Invest Ophthalmol Vis Sci* 1994; 1833.
28. Wilson DJ, Cuthbertson RA, Kijavin I. Apoptosis as the mechanism of photoreceptor cell death in experiment retinal detachment. (ARVO abstract) *Invest Ophthalmol Vis Sci* 1995; 36:S64.
29. Hafezi F, Weller M, Munz K, et al. Light-induced apoptosis in the rat retina in vivo: threshold, time course and potential regulators. *Invest Ophthalmol Vis Sci* (ARVO abstract) 1995; 36:S917.
30. Lai WW, Chang CJ, Abler AS, et al. A comparative study of apoptosis of photoreceptor cells following light injury to four strains of inbred albino rats. (ARVO abstract) *Invest Ophthamol Vis Sci.* 1995; 36:S63.

DISCUSSION

DR ALAN H. FRIEDMAN. Apoptosis (programmed cell death) occurs in response to a variety of stimuli and is associated with DNA strand breaks. Apoptosis differs from necrosis in several basic features. In necrosis, cells die from sudden and severe injury as caused, for example, by ischemia. In the ischemic process, the plasma membrane is the primary site of damage. This leads to swelling of cytoplasmic organelles and cells followed by rupture. Inflammation is an intrinsic component of necrosis, while DNA fragmentation is inconstantly present. Apoptosis is a markedly different physiologic process, in which cells die as a result of normal embryologic development, deprivation of hormonal support, and inflammatory mediators. The basic events of apoptosis, in contradistinction to necrosis, commence in the nucleus rather than the plasma membrane. Ultrastructurally, this is seen as dense chromatin condensation, nucleolar disintegration, reduction in nuclear size, invaginations in nuclear membranes, budding of nuclei and cytoplasm into apoptotic bodies, cell shrinkage, and loss of normal intercellular contacts. Apoptotic cells are phagocytosed by adjacent cells. Endonuclease activation, often due to calcium ion influx, degrades DNA into fragments of a predictable size.

In the study as presented here, Dr Tso and coworkers studied eyes obtained from autopsy and surgical enucleations due to a variety of pathologic conditions. They utilized a well-described technique: the TdT-mediated biotin-dUTP nicked-end labelling (TUNEL) technique for apoptosis detection.

TUNEL-positive labelling was observed in some of the eyes with pathologic myopia, traumatic retinal detachment, and age-related macular degeneration, particularly in photoreceptor nuclei. Little, if any, TUNEL-positive staining was observed in eyes with retinal detachment secondary to choroidal malignant melanoma, lattice degeneration, and paving stone degeneration.

The authors' results are interesting and raise several intriguing questions. Are the findings relevant? It may be that the negative findings in some of their cases may represent an inability of the TUNEL technique, which marks damaged DNA, to detect a process that takes place early in apoptosis and may have concluded by the time the study was done. It is well known that apoptotic cells are removed quickly by scavenging neighbor cells or macrophages. Do normal eyes show apoptosis? Sequential studies counting the number of apoptotic bodies may be pertinent. How long did the autopsy eyes sit before they were studied? Is this a factor in finding positive or negative results? There are other markers of apoptosis such as *in situ* end labeling, which is specific for apoptosis and helps to quantify the changes.

This interesting paper has furthered our understanding of some of the basic life processes.

MARK TSO, M.D. I would like to thank Dr. Friedman for his very insightful comments.

To rule out post-mortem changes and false positive labelling of TUNEL technique, we enucleated an eye from a 65-year-old man and delayed fixation for 72 hours at room temperature. None of the post-mortem antolytic retinal cells labelled with the TUNEL technique.

Furthermore, we created animal models of traumatic retinal detachment in rats. The retinal tissues were studied by TUNEL technique and distinct labelling of the photoreceptors was observed. We further examined the retinal DNA by agarose gel electrophoresis and noted distinct ladder pattern suggesting apoptosis. So we believe that the TUNEL labelled cells of the retina in this study were, indeed, undergoing apoptosis.