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Photoreceptor decay over time and apoptosis in experimental retinal detachment

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Abstract ● Background: Data are scarce on the actual rate and mode of outer nuclear layer decay in retinal detachment (RD). We used an experimental rabbit model to assess the presence of apoptosis and rate of photoreceptor death following RD. This model included the creation of localized and stable retinal blebs, while controlling for any decline of retinal elevation over time. ● Methods: RD was produced in New Zealand white rabbits by injecting 0.05 ml of 15% sodium hyaluronate (Healon GV) under the neural retina using a microsurgical technique. Animals were killed at 1, 2, 4, 7, 14 and 29 days. Retinal tissue was processed for light and electron microscopy and for in situ end labeling of fragmented DNA using a modification of the TUNEL technique. Photoreceptor cell nuclei were counted in the RD areas of maximum retinal elevation of 28 eyes, and an additional 4 eyes were used for nick end labeling. ● Results: Positive DNA nick end labeling, ultrastructural features and absence of necrotic cells indicated apoptotic photoreceptor cell death. Also, there was a rapid, almost linear elimination of photoreceptor nuclei over time. At 14 days only half of the number of nuclei were discernible, while approximately one tenth remained after 29 days. There was a statistically significant, but minimal decline in RD height over the 4 weeks of study. ● Conclusion: Following experimental RD in rabbits, apoptotic cell death is associated with an almost linear elimination of photoreceptor cells over time. The use of highly viscous sodium hyaluronate in separating the neural retina from the retinal pigment epithelium allows the RD to maintain a nearly constant height over a period of 4 weeks.

Introduction

It has long been known that retinal detachment (RD) is associated with diverse cellular pathology, such as proliferation of Müller cells and retinal pigment epithelium and, in contrast, deterioration or loss of photoreceptor cells [4]. Whereas degeneration of photoreceptor outer segments may be reversible following reattachment [5, 16, 21, 25], loss of cell nuclei indicates a terminal event causing irrevocable damage to the visual function. However, little is known about the actual rate of photoreceptor loss following RD.

The mechanism of photoreceptor cell elimination after RD is currently being elucidated, and while apoptosis is now believed to cause photoreceptor cell death in diverse degenerative retinal disease [2, 7, 9, 30, 32, 33, 35], some recent data also indicate the presence of apoptosis after traumatic RD in humans [8] and in experimental RD in cats [11] and rhesus monkeys [38].

It is conceivable that a well-established retinal circulation may prevent pathological changes from occurring in the inner layers of the neural retina, but the potential effect on the outer retinal layers remains unclear. However, oxygen profiles in the cat retina indicate that 90% of

the oxygen consumed by the photoreceptors comes from the choroid, the rest coming from the retinal circulation [22]. In contrast to feline and human retina, photoreceptor survival in the rabbit exclusively depends upon the retinal pigment epithelium and choroidal circulation. Thus, the avascular rabbit retina offers a pure and simple experimental model in which to assess photoreceptor loss after RD.

Materials and methods

Thirty-four eyes of 17 young (New Zealand) white rabbits (aged 7–9 weeks and weighing approximately 2 kg) underwent surgery under general anesthesia using intramuscular injections of ketamine hydrochloride 35 mg/kg (Ketalar, Parke-Davis, Morris Plains, N.J.), USA) and xylazine 5 mg/kg (Rompun, Bayer, Leverkusen, Germany). A sclerotomy was cut 1–2 mm from the limbus. A glass micropipette with a tapered tip (about 0.1 mm in diameter) was inserted into the vitreous and through the medullary ray of the neural retina about 1 disc diameter from the optic disc using an operating microscope; 0.05 ml of a 1.5% solution of sodium hyaluronate (Healon GV, Pharmacia, Uppsala, Sweden) was injected into the subretinal space. Reproducible and circumscribed blebs of RD were created which remained essentially stable during the time of study (29 days). Approval was obtained from the ethics committee of the Karolinska Institute, Stockholm, Sweden. Principles of laboratory animal care (NIH publication No. 86-23, revised 1985) were followed.

The animals were killed at 1, 2, 4, 7, 14 and 29 days and the eyes enucleated. Two eyes did not contain adequate blebs, but the remaining globes were immediately fixed for 24 h in ice-cold 4% buffered glutaraldehyde (28 eyes) or in 10% formaldehyde (four eyes). After fixation and gross examination, each RD was divided in half and embedded in paraffin or Epon. Step-wise sections were cut at 4 μm from the paraffin blocks, stained with hematoxylin-eosin and used for the counting of photoreceptor nuclei. Similarly, semithin (2 μm) sections were cut from all Epon blocks and stained with toluidine blue. Epon-embedded tissue was then routinely processed for transmission electron microscopy as appropriate.

The total number of photoreceptor nuclei per 0.9-mm retinal strip were counted at magnification ×640 in detached areas of 28 eyes, taking care to include the area of maximum retinal elevation (vertex area) of the RDs. To indicate the area counted on the specimen, we used a calibrated eye-piece microgrid for the central part (0.15 mm^2) of six fields. To minimize bias, the examiner was masked as to the duration of RD. Morphologically abnormal photoreceptor nuclei were included, whereas occasional apoptotic bodies were discarded. Similarly, cells extruded from the outer nuclear layer into the subretinal space were excluded from the counts.

Four eyes were used for the detection of apoptosis using the commercially available ApopTag modification (Oncor, Gaithersburg, MD, USA) of the terminal dUTP nick end labeling (TUNEL) technique [14]. Briefly, sections were cut at 4 μm from formaldehyde-fixed, paraffin-embedded tissue, deparaffinized and rehydrated through graded alcohols. Protein was then digested by treating the tissue slides with pronase at 37° C for 5 min, whereafter endogenous peroxidase was quenched by 2% hydrogen peroxide for 5 min. The ApopTag processing then included the application of two drops of an equilibration buffer directly to each specimen followed by 10–15 s incubation beneath a plastic coverslip. After removal of the coverslip, 54 μl of working strength deoxynucleotidyl transferase was applied directly on each specimen, whereafter tissue slides were incubated beneath a plastic coverslip in a humidified chamber at 37° C for 1 h. Following removal of the plastic coverslip, each specimen was put in a Coplin jar containing pre-warmed working strength stop/wash buffer and incubated for 30 min at 37° C in a humidified chamber. Again, the coverslips were removed and two drops of anti-dioxygenin peroxidase applied to each specimen. The specimens were then incubated in a humidified chamber for 30 min at room temperature. Finally, sections were stained with 3-amino-9-ethylcarbazole, counterstained with weak hematoxyline and mounted.

Positive controls – formaldehyde-fixed, paraffin-embedded sections from female rodent mammary gland retrieved 3–5 days after the weaning of rat pups – were obtained from the manufacturer. These tissue slides were then included in the protocol outlined above. Negative controls were provided by substituting 32 μl of distilled water for deoxynucleotidyl transferase, as suggested by the manufacturer and others [19, 23, 34].

The inter-observer reproducibility was assessed using repeated counts by an independent observer masked to the duration of RD and the results of the initial observer. The initial observer repeated the cell counts at a later stage to assess intra-observer reproducibility.

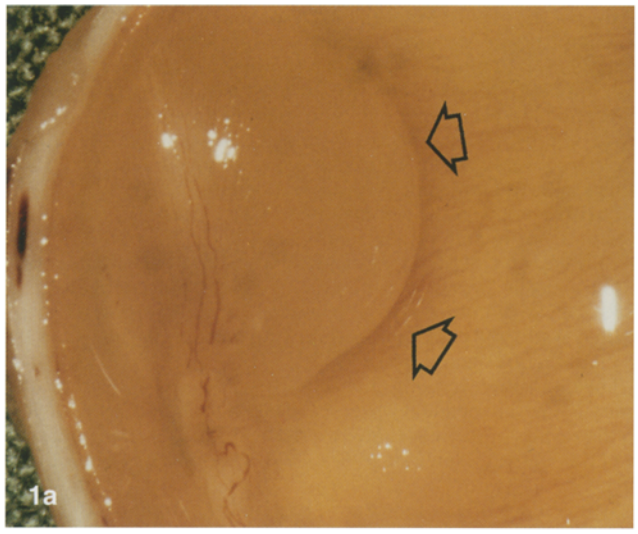
Scatterplots of photoreceptor loss and maximum RD height over time were arranged and linear regression was performed. The goodness-of-fit of a linear model for photoreceptor loss over time was then compared to that of an exponential model using analysis of variance. To control for inter-eye correlation [31], separate analyses were made for paired data (28 eyes of 17 rabbits) and for independent data (17 eyes of 17 rabbits). The level of statistical significance was set at 0.05. All calculations were computerized, based on the Complete Statistical System (Statistica), release 3.1 (Statsoft Inc, Tulsa, Okla, USA) and SPSS release 6.0 (SPSS, Chicago, Ill., USA).

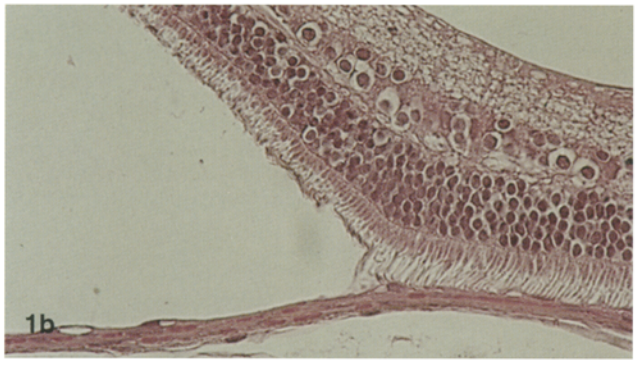
Results

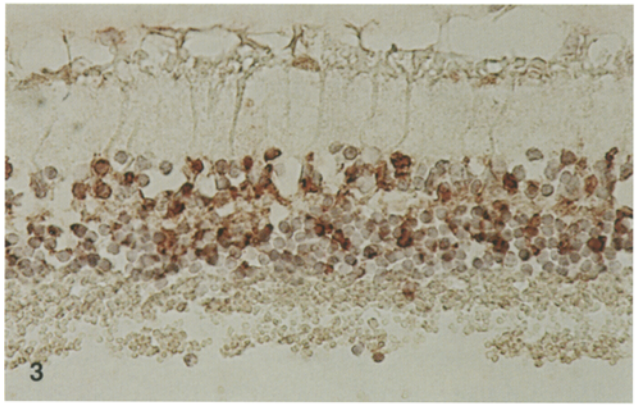
The experimentally induced RD consistently formed a round bleb of elevated retina distinct delineated from the attached retina (Fig. 1). Histopathological examination disclosed that, in the detached retina, the photoreceptor outer segments were destroyed, the inner segments severely damaged and the number of receptor cell nuclei reduced. These morphological features were more pronounced after prolonged RD (Fig. 2), but also varied considerably within each RD.

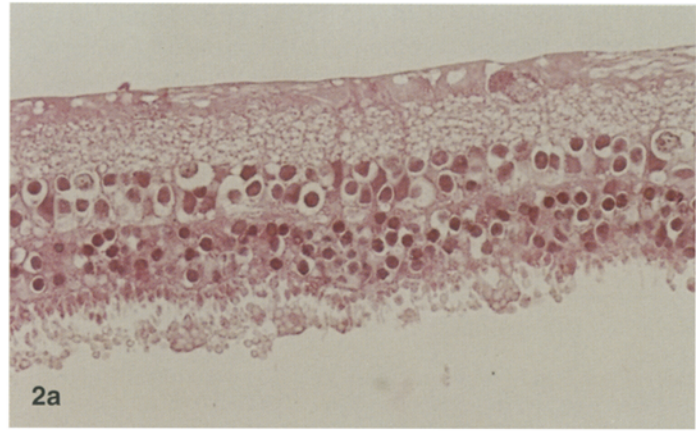
There was no inflammatory cellular response, and no macrophages were observed in the neural retina. Photoreceptor nuclei were occasionally extruded from the outer nuclear layer into the subretinal space. Generally, however, photoreceptor nuclei disappeared without leaving any remnants or cell necrosis. By light microscopy, a few apparently single apoptotic bodies were present in the outer nuclear layer after 4 days of RD. However, deoxyribonucleic acid (DNA) nick end labeling disclosed large numbers of positively staining photoreceptor nuclei following 2 days of RD (Fig. 3). Similarly, electron microscopy revealed photoreceptor cell shrinkage and condensation of hyperchromatic nuclei, consistent with apoptosis (Fig. 4).

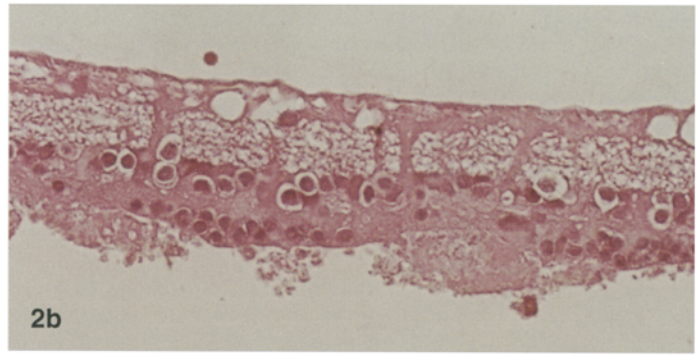
The receptor cell degeneration was rapid. A significant decrease in the number of nuclei was demonstrated within 2 days after surgery and the number of photore-











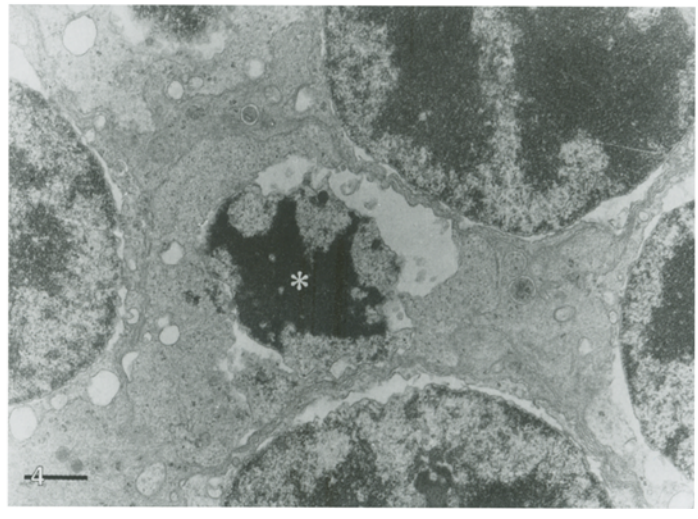


Fig. 1 **a** Photograph of gross specimen showing a circumscribed retinal detachment (*arrows*) adjacent to the medullary ray. **b** Microphotograph of periphery of retinal detachment featuring a distinct transition between detached and attached retina

Fig. 2a, b Microphotographs showing vertex of retinal detachment with **a** 2 days' and **b** 7 days' duration, respectively. Note the decline in the number of photoreceptor cell nuclei over time

Fig. 3 DNA nick end labeling (ApopTag) of photoreceptor nuclei in retina detached for 2 days. Numerous nuclei stained with 3-amino-4-ethylcarbazole (*red*) indicate the presence of extensive apoptosis

Fig. 4 Electron micrograph showing shrinkage of photoreceptor cell with condensation of the hyperchromatic nucleus (*asterisk*) consistent with apoptotic cell death. *Bar* 1 μm

ceptor cell nuclei declined gradually over time. After 14 days half of the number of nuclei remained, while at 29 days photoreceptor cell death was very extensive and only about one tenth of the original number of nuclei were still discernible (not shown).

The decrease in the number of photoreceptor cell nuclei over time appeared almost linear (Fig. 5). Similarly, analysis of variance showed that the goodness-of-fit of the linear regression model was satisfactory, the sums of squares indicating that 76% (3,550,372.7/4,664,993.5) of the total variation could be explained by the regression (Table 1). However, when the same data set was entered

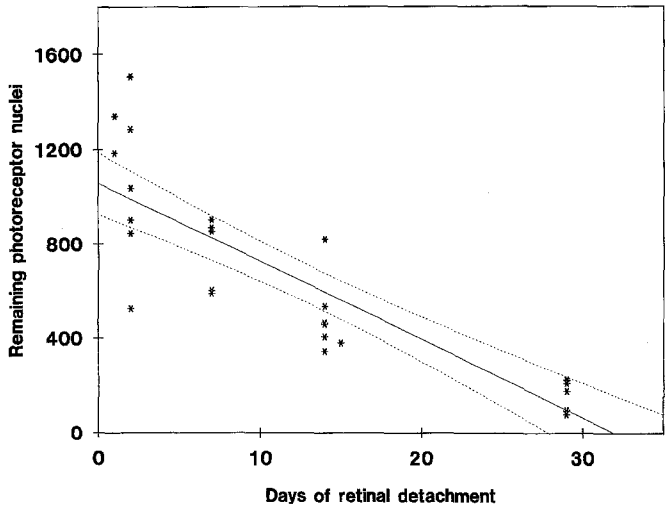


Fig. 5 Linear regression of photoreceptor nuclei per 0.9-mm retinal strip on retinal detachment time. The estimated slope (*solid line*) is statistically significant (standard error 3.6; t statistic=9.1; $P<10^{-8}$). The 95% confidence interval of the regression line is included (*broken lines*)

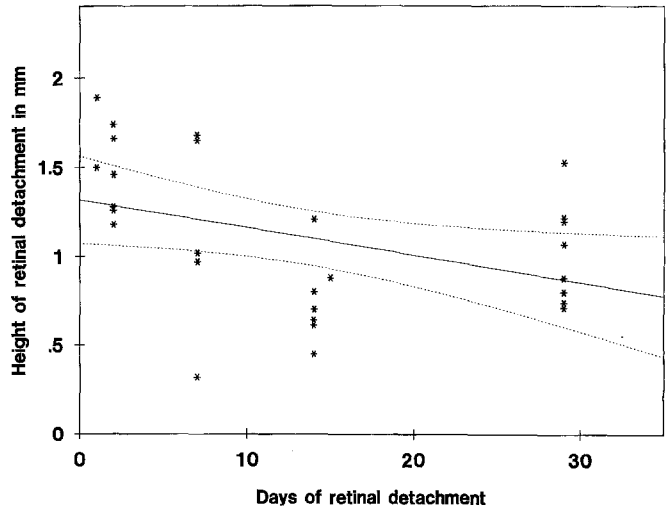


Fig. 6 Linear regression of retinal detachment height on retinal detachment time. The regression line (*solid line*) is statistically significant (standard error 0.0069; t statistic=2.2; P=0.034). The 95% confidence interval of the regression line is included (*broken lines*)

Table 1 Analysis of variance for linear model of photoreceptor loss over time (paired data for 28 eyes of 17 rabbits)

Source of variation	df	Sums of squares	Mean squares	F	P
Regression	1	3,550,372.7	3,550,372.7	83	$<10^{-8}$
Residual	26	1,114,620.8	42,870.0		
Total	27	4,664,993.5			

Table 2 Analysis of variance for exponential model of photoreceptor loss over time (paired data for 28 eyes of 17 rabbits)

Source of variation	df	Sums of squares	Mean squares	F	P
Regression	1	18,513,858	18,513,858	155	$<10^{-11}$
Residual	26	3,103,265	119,356		
Total	27	21,617,123			

Table 3 Analysis of variance for linear model of photoreceptor loss over time (independent data for 17 eyes of 17 rabbits

Source of variation	df	Sums of squares	Mean squares	F	P
Regression	1	2,087,057.4	2,087,057.4	35	$<10^{-4}$
Residual	15	892,751.7	59,516.8		
Total	16	2,979,809.1			

Table 4 Analysis of variance for exponential model of photoreceptor loss over time (independent data for 17 eyes of 17 rabbits)

Source of variation	df	Sums of squares	Mean squares	F	P
Regression	1	11,739,258	11,739,258	72	10^{-6}
Residual	15	2,449,616	163,308		
Total	16	14,188,874			

into an exponential model the sums of squares suggested that 86% of the total variation could be explained by the regression (Table 2). Ophthalmic data frequently have a strong inter-eye correlation [31], and to adjust for any correlation between fellow eyes, the calculations were repeated for a set of eyes comprising one randomly selected eye from each of the 17 animals. When using this unpaired data set, both regression models retained statistical significance. However, the exponential model maintained a slightly better goodness-of-fit, accounting for 82% of the total variation, compared to 70% when using a linear model (Tables 3, 4).

The retinotomy at the medullary ray was almost self-sealing and there appeared to be little, if any, leakage or resorption of the highly viscous sodium hyaluronate solution. Correspondingly, the RDs showed only minimal reduction in height (Fig. 6). While this decline was statistically significant (P=0.034), analysis of variance suggested that only 16% could be attributed to the regression using a linear model (not shown).

The intra-observer reproducibility of photoreceptor nuclei counts was satisfactory, showing only minimal variation. Statistical analysis revealed no significant difference between two masked counts (Fig. 7). Moreover,

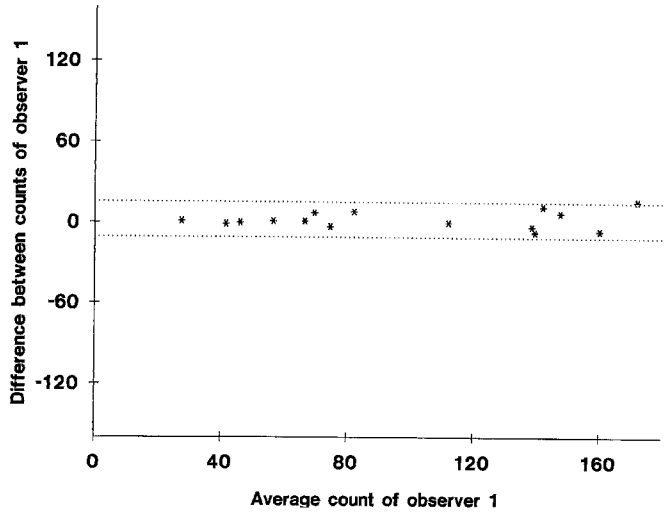


Fig. 7 Intra-observer reproducibility of photoreceptor nuclei assessments. Observer 1 counted 15 retinal strips of 0.15 mm length at magnification ×640 twice in a masked fashion. The differences between the two counts are plotted against the mean count. The graph suggests minimal intra-observer variability. The 95% limit of agreement (−10.8 to +15.2) as defined by the mean (2.2) ± two standard deviations (SD=6.5) is indicated (*broken lines*). The hypothesis of zero bias was formally tested by the two-tailed *t*-test for paired data (P=0.21), indicating no significant difference between the two counts

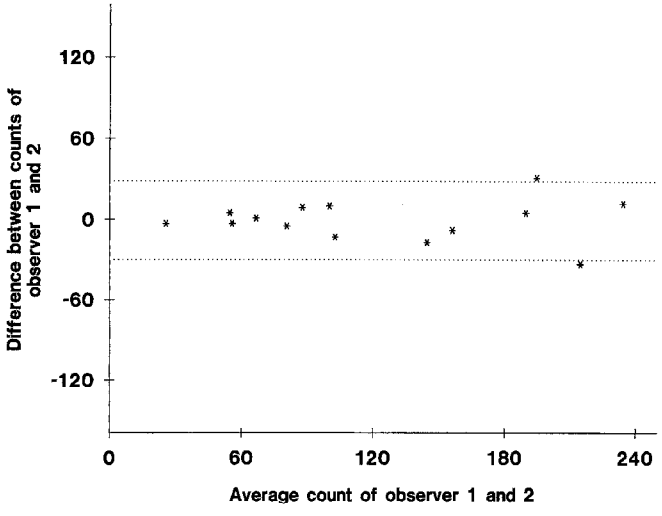


Fig. 8 Inter-observer reproducibility of photoreceptor nuclei counts. Observer 1 and observer 2 each counted 15 retinal strips of 0.15 mm length at magnification ×640 in a masked fashion. The differences between the two assessments are plotted against the mean count of the two observers. The graph suggests no significant inter-observer variability. The 95% limit of agreement (−30.0 to +28.4) as defined by the mean (−0.8) ± two standard deviations (SD=14.6) is indicated (*broken lines*). The hypothesis of zero bias was confirmed by the two-tailed *t*-test for paired data (P=0.84), indicating no significant difference between the two observers

the inter-observer variability was minimal and the difference did not reach statistical significance (Fig. 8).

Discussion

In this experimental model, using a viscous solution of sodium hyaluronate to create an RD, there is an almost linear decay of the number of photoreceptor nuclei over time; after 4 weeks of RD the reduction of photoreceptor nuclei was approximately 90%. Most previous data on experimental RD derive from animal studies. In owl monkeys (*Aotus trivirgatus*) degeneration of photoreceptor outer segments and the formation of intraretinal cystoid spaces were observed within 2 weeks' duration of RD [20, 24]. The investigators reported little evidence of cell death in the outer nuclear layer of the retina before 14 weeks. While experimental studies on RD in rabbits have indicated severe loss of photoreceptor cells after 4 months [13, 28], a significant decrease of the number of photoreceptor nuclei was found in the feline retina after 1 month [12]. Our results indicate that the photoreceptor loss of the rabbit is extensive after only 4 weeks of an RD with an elevation of only 1.0–1.5 mm.

There may be several explanations for this difference. It is reasonable to assume that a highly viscous (1.5%) solution of sodium hyaluronate impairs the convection of fluid and creates a relative barrier to the diffusion of several vital substances from the choroid and the retina pigment epithelium to the neurosensory retina. The diffusion rate of oxygen, nitrogen and glucose, however, does not seem to be greatly reduced in a solution of sodium hyaluronate [17, 26]. However, the diffusion of large molecular substances is probably reduced more. In the opposite direction from the neural retina to the choroid, the diffusion of lactate [36] may also be critically impaired, yielding a low pH in the neural retina. These effects are likely to aggravate photoreceptor death in this experimental model as compared to the free diffusion of substances in the aqueous subretinal fluid.

It has been suggested that the RD height, i.e. the distance from the detached retina to the retinal pigment epithelium, will substantially influence photoreceptor degeneration [24]. Thus, the severity of the degenerative changes in the neural retina may vary in different experimental models depending upon the configuration of RD. In the present experimental model the initial RD height and the size and shape of the RD were well defined and remained essentially stable throughout the study period of 4 weeks. This is in striking contrast to some recent data using diverse fluids to separate the neural retina from the retinal pigment epithelium. When autologous aqueous humor, autologous vitreous and autoserum are injected into the subretinal space, the RD disappears within 24 h [1]. Similar to our study, other authors have injected a viscoelastic substance subretinally to create a

well-circumscribed RD [4, 11, 12], but few data are available on the shape and size of the bleb after prolonged detachment. In contrast to these reports, we used a more viscous solution of sodium hyaluronate (1.5% compared to 0.5%) to create a stable retinal bleb.

In apoptotic cell death single cells are deleted in the midst of living tissue [40]. These cells are disposed of very neatly by a process of specific recognition and phagocytosis, in contrast to the non-selective events involved in necrotic cell death [37]. Apoptosis originally was defined by morphological criteria, including cell volume loss, nuclear condensation and the formation of apoptotic bodies [18, 41], and later by characteristic degradation of the DNA to oligonucleosomal fragments recognizable by agarose gel electrophoresis [3, 27, 29]. Recently, techniques have been introduced whereby the DNA breaks may be recognized by in situ end labeling [14, 15, 19, 23, 34, 39]. Apoptotic bodies are usually seen only for a few hours before phagocytosis [6] , and in situ end labeling will not just confirm the presence of DNA fragmentation, but may also identify a larger proportion of cells entering apoptosis. Conceivably, DNA oligonucleosomal fragmentation may occur in the absence of apoptosis, and the validity of gel electrophoresis for identifying apoptosis has recently been challenged [10]. Recent findings suggest that in situ nick end labeling is more sensitive than agarose gel electrophoresis in detecting internucleosomal DNA cleavage [35]. It is currently recommended that the recognition of apoptosis should be based both on DNA degradation patterns and ultrastructural features [10].

Therefore, our present data are based upon a combination of in situ nick end labeling and electron microscopy to unequivocally demonstrate the presence of apoptosis. The apoptotic photoreceptor loss in RD is further supported by the absence of significant retinal necrosis. The process of retinal degeneration after RD appears to occur through the focal and continuous elimination of photoreceptor cells, consistent with apoptosis being a major contributor, in accordance with earlier studies [8, 11, 38].

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References

1. Abe T, Yoneya S, Mori K, Hayashi N, Isono H (1994) Reaction of the retina to injected subretinal fluids. J Jpn Ophthalmol Sci 98:453–462
2. Abler AS, Chang FJ, Fu J, Tso MOM (1994) Photic injury triggers apoptosis of photoreceptor cells (abstract). Invest Ophthalmol Vis Sci 35:1517
3. Alnemri ES, Litwack G (1990) Activation of internucleosomal DNA cleavage in human CEM lymphocytes by glucocorticoid and novobiocin. J Biol Chem 265:17323–17333
4. Anderson DH, Stern WH, Fisher SK, Erickson BH, Borgula GA (1983) Retinal detachment in the cat. The pigment epithelial-photoreceptor interface. Invest Ophthalmol Vis Sci 24:906–926
5. Anderson DH, Guerin CJ, Erickson PA, Stern WH, Fisher SK (1986) Morphological recovery in the reattached retina. Invest Ophthalmol Vis Sci 27:168–183
6. Bursch W, Paffe S, Putz B, Barthel G, Schulte-Hermann R (1990) Determination of the length of the histological stages of apoptsis in normal liver and in altered hepatic foci of rats. Carcinogenesis 11:847–853
7. Chang C-J (1994) Apoptosis in retinal photoreceptor degeneration. Thesis, University of Illinois at Chicago, Chicago, Illinois
8. Chang C-J, Lai WW, Edward DP, Tso MOM (1995) Apoptotic photoreceptor cell death after retinal detachment in humans. Arch Ophthalmol 113:880–886
9. Chang G-Q, Hao Y, Wong F (1993) Apoptosis: final common pathway of photoreceptor death in rd, rds and rhodopsin mutant mice. Neuron 11:595–605
10. Collins RJ, Harmon BV, Gobé GC, Kerr JFR (1992) Internucleosomal DNA cleavage should not be the sole criterion for identifying apoptosis. Int J Radiat Biol 61:451–453
11. Cook B, Lewis GP, Fisher SK, Adler R (1995) Apoptotic photoreceptor degeneration in experimental retinal detachment. Invest Ophthalmol Vis Sci 36:990–996
12. Erickson PA, Fisher SK, Anderson DH, Stern WH, Borgula GA (1983) Retinal detachment in the cat: the outer nuclear and outer plexiform layers. Invest Ophthalmol Vis Sci 24:927–942
13. Foulds WS (1963) Experimental retinal detachment. Trans Ophthalmol Soc UK 83:153–170
14. Gavrieli Y, Sherman Y, Ben-Sasson SA (1992) Identification of programmed cell death in situ via specific labelling of nuclear DNA fragmentation. J Cell Biol 119:493–501
15. Gorczyca W, Bruno S, Darzynkiewicz RJ, Gong J, Darzynkiewicz Z (1992) DNA strand break occurring during apoptosis: their early in situ detection by the terminal deoxynucleotidyl transferase and nick translation assays and prevention by serine protease inhibitors. Int J Oncol 1:639–648
16. Guerin CJ, Lewis GP, Fisher SK, Anderson DH (1993) Recovery of photoreceptor outer segment length and analysis of membrane assembly in regenerating primate photoreceptor outer segments. Invest Ophthalmol Vis Sci 34:175–183
17. Hadler NM (1980) Enhanced diffusivity of glucose in a matrix of hyaluronic acid. J Biol Chem 255:3532–3535
18. Kerr JFR, Wyllie AH, Currie AH (1972) Apoptosis, a basic biological phenomenon with wider implications in tissue kinetics. Br J Cancer 26:239–245
19. Kong J, Ringer DP (1995) Quantitative in situ image analysis of apoptosis in well and poorly differentiated tumors from rat liver. Am J Pathol 147:1626–1632
20. Kroll AJ, Machemer R (1968) Experimental retinal detachment in the owl monkey. III. Electron microscopy of retina and pigment epithelium. Am J Ophthalmol 66:410–427

21. Kroll AJ, Machemer R (1969) Experimental retinal detachment in the owl monkey. V. Electron microscopy of the reattached retina. Am J Ophthalmol 67: 117–130
22. Linsenmeier RA, Braun RD (1992) Oxygen distribution and consumption in the cat retina during normoxia and hypoxemia. J Gen Physiol 99: 177–197
23. Liu Q, Oshima K, Shinohara T, Kikuchi M (1995) Apoptosis in histiocytic necrotizing lymphadenitis. Pathol Int 45: 729–734
24. Machemer R (1968) Experimental retinal detachment in the owl monkey. II. Histology of retina and pigment epithelium. Am J Ophthalmol 66: 396–410
25. Machemer R (1968) Experimental retinal detachment in the owl monkey. IV. The reattached retina. Am J Ophthalmol 66: 1075–1091
26. McCabe M, Laurent TC (1975) Diffusion of oxygen, nitrogen and water in hyaluronate solutions. Biochim Biophys Acta 399: 131–138
27. McConkey DJ, Aguilar-Santelises M, Hartzell P, Eriksson I, Mellstedt H, Orrenius S, Jondal M (1991) Induction of DNA fragmentation in chronic B-lymphocytic leukemia cells. J Immunol 146: 1072–1076
28. Nakamura S (1965) Morphological observation of experimental retinal detachment of the retina. Part 1. Acta Soc Ophthalmol Jpn 69: 1329–1356
29. Newell MK, Haughn LJ, Maroun CR, Julius MH (1990) Death of mature T cells by separate ligation of CD4 and T-cell receptor antigen. Nature 347: 286–288
30. Portera-Cailliau C, Sung C-H, Nathans J, Adler R (1994) Apoptotic photoreceptor cell death in mouse models of retinitis pigmentosa. Proc Natl Acad Sci USA 91: 974–978
31. Ray WA, O'Day DM (1985) Statistical analysis of multi-eye data in ophthalmic research. Invest Ophthalmol Vis Sci 26: 1186–1188
32. Shahinfar S, Edward DP, Tso MO (1991) A pathologic study of photoreceptor cell death in retinal photic injury. Curr Eye Res 10: 47–59
33. Smith SB, Bora N, McCool D, Kutty G, Wong P, Kutty RK, Wiggert B (1995) Photoreceptor cells in the vitiligo mouse die by apoptosis: TRPM-2/clusterin expression is increased in the neural retina and in the retinal pigment epithelium. Invest Ophthalmol Vis Sci 36: 2193–2201
34. Su JH, Anderson AJ, Cummings BJ, Cotman CW (1994) Immunohistochemical evidence for apoptosis in Alzheimer's disease. Neuroreport 5: 2529–2533
35. Tso MOM, Zhang C, Ahler AS, Chang C-J, Wong F, Chang G-Q, Lam TT (1994) Apoptosis leads to photoreceptor degeneration in inherited retinal dystrophy of RCS rats. Invest Ophthalmol Vis Sci 35: 2693–2699
36. Wang I, Kondo M, Bill A (1997) Glucose metabolism in cat other retina: effects of light and hyperoxia. Invest Ophthalmol Vis Sci 38: 48–55
37. Williams GT (1991) Programmed cell death: apoptosis and oncogenesis. Cell 65: 1097–1098
38. Wilson DJ, Cuthbertson RA, Kljavin I (1995) Apoptosis as the mechanism of photoreceptor cell death in experimental retinal detachment. (abstract) Opthalmol Vis Sci 36:S64
39. Wisjman JH, Jonker RR, Keijzer R, van de Velde CJH, Cornelisse CJ, van Dierendonck JH (1993) A new method to detect apoptosis in paraffin sections: in situ end-labeling of fragmented DNA. J Histochem Cytochem 41: 7–12
40. Wyllie AH (1993) Apoptosis (the Frank Rose memorial lecture). Br J Cancer 67: 205–208
41. Wyllie AH, Kerr JFR, Currie AR (1980) Cell death: the significance of apoptosis. Int Rev Cytol 68: 251–306