PII: S1350-9462(98)00032-9

Mechanisms of Photoreceptor Death and Survival in Mammalian Retina

Jonathan Stone*, Juliani Maslim, Krisztina Valter-Kocsi, Kyle Mervin, Felicity Bowers, Yi Chu, Nigel Barnett, Jan Provis, Geoff Lewis, Steven K. Fisher, Silvia Bisti, Claudia Gargini, Luigi Cervetto, Saul Merin and Jacob Pe'er

NSW Retinal Dystrophy Research Centre, Department of Anatomy and Histology, University of Sydney, Sydney, Australia; Department of Anatomy and Histology, University of Sydney, Sydney, Australia; Institute for Neuroscience, University of California, Santa Barbara, USA; Dipartimento STBB, Universita' dei L'Aquila, L'Aquila, Italy; Istituto di Neurofisiologia, CNR, Pisa, Italy; Dipartimento di Psichiatria, Neurobiologia, Farmacologia e Biotecnologie, Universita' di Pisa, Pisa, Italy and Department of Ophthalmology, Hadassah Faculty of Medicine, Hadassah University Hospital, The Hebrew University of Jerusalem, Israel

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*Corresponding author. Fax: +0061-2-9351-6556; E-mail: jonstone@anatomy.usyd.edu.au

Progress in Retinal and Eye Research Vol. 18, No. 6, pp. 689 to 735, 1999

Printed in Great Britain
1350-9462/99/$ - see front matter

Abstract—The mammalian retina, like the rest of the central nervous system, is highly stable and can maintain its structure and function for the full life of the individual, in humans for many decades. Photoreceptor dystrophies are instances of retinal instability. Many are precipitated by genetic mutations and scores of photoreceptor-lethal mutations have now been identified at the codon level. This review explores the factors which make the photoreceptor more vulnerable to small mutations of its proteins than any other cell of the body, and more vulnerable to environmental factors than any other retinal neurone. These factors include the highly specialised structure and function of the photoreceptors, their high appetite for energy, their self-protective mechanisms and the architecture of their energy supply from the choroidal circulation. Particularly important are the properties of the choroidal circulation, especially its fast flow of near-arterial blood and its inability to autoregulate. Mechanisms which make the retina stable and unstable are then reviewed in three different models of retinal degeneration, retinal detachment, photoreceptor dystrophy and light damage. A two stage model of the genesis of photoreceptor dystrophies is proposed, comprising an initial "depletion" stage caused by genetic or environmental insult and a second "late" stage during which oxygen toxicity damages and eventually destroys any photoreceptors which survive the initial depletion. It is a feature of the model that the second "late" stage of retinal dystrophies is driven by oxygen toxicity. The implications of these ideas for therapy of retinal dystrophies are discussed.

1. DURABILITY AND FRAGILITY

It is easy to be impressed by both the durability and the fragility of mammalian photoreceptors. In phylogenetic terms their design—each is a neurone of the central nervous system in which the cilium is specialised to bear photosensitive pigments and signal their breakdown—is considered to have been conserved throughout the evolution of vertebrates, thus over hundreds of millions of years. In ontogenetic terms the photoreceptor shares the ability of all central nervous system neurones to survive and function for the full life of the whole animal, in long-lived species like *Homo sapiens* for seven, eight or nine decades.

The mechanisms which give the photoreceptor its evolutionary and individual durability would seem therefore to deserve close study. In practice however photoreceptors have proved more fragile than other CNS neurones, more vulnerable to small mutations in their proteins, more sensitive to environmental damage. It has therefore been the *fragility* of photoreceptors which has attracted most study, particularly because their degeneration leaves the major sensory deficit of blindness in diseases such as retinitis pigmentosa and macular degeneration.

1.1. Sources of Fragility

In seeking the sources of this fragility, attention turns quickly to the specialisations of the photoreceptor. Its durability it shares with other CNS neurones. It is specialisations—the building, the maintenance and the energy demands of the photoreceptive outer segment—which equip the photoreceptor for its function and make it vulnerable.

1.1.1. *The fragility of the outer segment membrane*

The membrane of the outer segment appears to be less stable than the membranes of other CNS neurones, and damage to it is a sensitive index of stress, for example by bright light (Penn and Anderson, 1991). Another index of the instability of outer segment membrane is its fast turnover; it is continuously replaced from its cilial end and discarded from its tip (Young, 1974; Anderson *et al.*, 1978; LaVail, 1983). The rate of this turnover (the entire outer segment of rat rods is replaced every two weeks) seems to be unique to the photoreceptor and it requires the tips of photoreceptors to be apposed to an efficiently phagocytotic epithelium. This requirement poses a major problem for retinal architecture in the vertebrate eye, and the solution which has evolved to this problem is a major source of the vulnerability of photoreceptors.

1.1.2. *The need to invert the retina*

The requirement that the photoreceptive outer segments of photoreceptors be apposed to a phagocytotic membrane is met in the vertebrate eye by a combination of evolutionary and ontogenetic "decisions" which invert the retina, locating the photoreceptive elements on its outer surface, away from the pupil. A more logical retinal structure is found in the one invertebrate group with an optical eye, the cephalopods (squids, cuttlefish, octopi), whose photoreceptors have evolved a photoreceptive element (the rhabdome) quite different from the vertebrate outer segment. The rhabdome does not require servicing by a phagocytotic membrane and the photoreceptors lie at the inner surface of the retina with their rhabdomes pointed towards the pupil, directly accessible to light (Young, 1989). In the vertebrate retina, by contrast, the photoreceptors form a layer at the outer aspect of the retina, and orient their outer segments outwards, so that they can "stick into" and be serviced by the retinal pigment epithelium (RPE) (Steinberg *et al.*, 1976; Anderson *et al.*, 1978; LaVail, 1983). The vertebrate eye forms images on the outer segments, and light forming the image must pass through all the layers of the retina, from axons to inner segments, before reaching that image plane. An effective phagocytotic relation is thus established between the tips of the outer segments and the RPE, but at the "cost" of inverting the retina.

The organisation of the vertebrate retina is highly successful but it also fails, as in the photoreceptor dystrophies. We argue here that the inversion of vertebrate retina required the evolution of extreme patterns of energy supply, in which lies one cause of the failures.

1.1.3. *The need for energy and the need to be avascular*

For reasons reviewed elsewhere (Ames *et al.*, 1992; Demontis *et al.*, 1997), the function and maintenance of the outer segment require prodigious amounts of energy in the form of phosphorylated nucleotides, particularly ATP. Photoreceptors generate ATP principally (Winkler, 1995) from glucose (as do other neurones), but their consumption of oxygen for the oxidative metabolism of glucose and their consumption of glucose by the anaerobic process of glycolysis are both extremely high (Section 3.1). The function of photoreceptors (Section 3.1.1, Section 3.1.2) and eventually their survival (Section 2.3) depend on the supply of glucose and oxygen in large quantities.

If the photoreceptors were supplied with nutrients by vessels coursing among them close to their inner segments (their great energy sinks), the vessels would (because of the inversion of retinal structure) create gaps in the photoreceptor array and cast shadows on the outer segments. Presumably to prevent such shadows and gaps, blood vessels are strictly excluded by a still unknown mechanism from the outer half of the retina, creating the paradox that the most energy-hungry region of the central nervous system is the only region to lack intrinsic vessels. Their energy needs have been met by the evolution of a dedicated vascular bed, the choroid, located immediately external to the RPE. Glucose and oxygen reach the photoreceptors by diffusion from the choroidal circulation, across the RPE and its prominent basement membrane (Bruch's membrane). The logistical challenge of meeting their high

energy demand by diffusion from a distance is extreme however, and to meet it the choroidal circulation has evolved extreme properties.

1.1.4. *The choroidal circulation: Achilles' heel of the eye?*

The choroid is an anatomically profuse plexus of vessels whose capillaries (collectively termed the choriocapillaris) are the most permeable of any circulation, being 30 times more permeable than capillaries of skeletal muscle, and five times more permeable than the capillaries of the kidney (Bill *et al*., 1980). Supplied by several ciliary arteries and drained by several "vortex" veins, the choroid's throughput of blood is (volume for weight of tissue supplied) 40–50 times that of the retinal circulation (which supplies the inner half of the retina) or cerebral circulation, and four times faster than the flow through the kidney cortex (Alm and Bill, 1972). Flow is so fast through the choroid that the blood in the vortex veins contains near-arterial levels of oxygen (Bill *et al*., 1983). The teleologically valuable result of this arrangement is that the choriocapillaris bathes the outer aspect of the retina with near-arterial levels of nutrients, which diffuse to the photoreceptors to fuel their prodigious metabolism. Bill *et al*. (1983) further suggested that the fast flow of the choroidal circulation also serves to remove heat generated by that metabolism.

The problem with the choroidal circulation is that it does not regulate itself well. Presumably because its capillaries do not lie in the tissue they supply, they cannot "autoregulate", i.e., they cannot adjust their delivery of blood to what is happening in the tissue they are supplying (Bill and Sperber, 1990). One consequence is that when the oxygen consumption of the photoreceptors falls, as when the rods light adapt, oxygen tension in the ONL rises sharply, from near hypoxia to 30 mmHg (Linsenmeier, 1986; Wolbarsht *et al*., 1987; Brown *et al*., 1996), so that photoreceptors experience a marked daily "swing" in oxygen levels which does not occur in any other tissue.

The inability of the choroidal circulation to autoregulate is the source of much pathology of the retina. If blood oxygen levels rise, for example in a baby given oxygen to relieve respiratory distress, oxygen floods from the choroid across the retina. Oxygen in deficit (i.e., hypoxia) is a powerful and normal developmental stimulus, e.g., for angiogenesis in neonatal retina (Section 2.2.5), and the (unintended) elimination of retinal hypoxia in neonates, by the uncontrolled flow of oxygen from the choroid, was the major cause of the disease retinopathy of prematurity (reviewed Chan-Ling and Stone, 1993; Stone and Maslim, 1997). Oxygen in excess is also a powerful toxin and we argue below (Section 4.2.4) that the chronically unregulated flow of oxygen from the choroid to outer retina is a major cause of the late stages of photoreceptor dystrophies.

1.2. The Normality and Abnormality of Photoreceptor Death

Although millions of photoreceptors in each eye last the full length of human life, millions do not. Even in normal human eyes photoreceptors die throughout life (Dorey *et al*., 1989; Gao and Hollyfield, 1992). The data of Gao and Hollyfield indicate that at least from the late teen years and probably earlier, every human eye loses hundreds of thousands of photoreceptors every year. Correspondingly, our vision slowly fades. Even after allowing for age-related changes in optical clarity, thresholds in sensitivity, acuity and colour tasks rise with age after the sixth decade (Sloane *et al*., 1988; Knoblauch *et al*., 1987). Because of the very high numbers of photoreceptors initially generated (over 100 million in each young human eye) good retinal performance can persist into advanced age, but if we live long enough, blindness becomes part of the "oblivion" of advanced age—"...sans teeth, sans eyes, sans everything" (*As You Like It II, vii, 139*).

This continuing normal photoreceptor loss is accelerated by "scores of mutations in dozens of genes" (Dryja and Berson, 1995), causing the blindness of conditions known as retinitis pigmentosa (RP). No other neurone is so vulnerable to so many small mutations, and the large cohort of RP cases with no family history suggest that the normal rate of photoreceptor loss can also be accelerated by environmental factors. We next

review some of the factors that make photoreceptors both durable and vulnerable.

2. PHOTORECEPTOR DEVELOPMENT: OVERPRODUCTION, CULLING AND A CRITICAL PERIOD

2.1. Developmental Death of Photoreceptors

The generation of photoreceptors and other retinal neurones has been reviewed elsewhere (Stone, 1987; Robinson, 1991; Rapaport and Vietri, 1991; LaVail *et al.*, 1991). Emphasis is given here to a period of death among photoreceptors which appears to be a normal part of their development. During this period (we suggest) the population of photoreceptors is culled from an initial excess to a level appropriate for adult life. Evidence of a cull is reviewed here because regulation of the cull is a central factor in the genesis of photoreceptor dystrophies.

2.1.1. *Evidence of overproduction and culling*

Throughout the central nervous system the genesis of cells is followed by a period of death among the newly born cells. Within many subpopulations of CNS neurones, the death begins before the genesis is complete and extends for some days after its end. Most reviewers, for example Cowan (1973), interpret this close linkage of birth and death as a sequence of overproduction and culling to adult levels. In the CNS (including the retina) the culling is seen as a scattering of degenerating cells among probably all subpopulations of neurones, typically becoming prominent in late foetal life and disappearing during early postnatal development (e.g., Ferrer *et al.*, 1990, 1994; Young, 1984; Provis and Penfold, 1988; Harman *et al.*, 1989; Hamburger and Oppenheim, 1982). All cellular layers of the retina, and probably all classes of neurones found in those layers, are affected (reviewed in Robinson, 1991).

Several non-exclusive ideas of the significance of developmental overproduction and culling have been proposed. Overproduction could serve an evolutionary role, providing a reserve of neurone numbers whose modulation could provide selective advantage (Williams *et al.*, 1993). Alternatively or as well, the culling of overproduced neurones by specific rules could create some of the specificity of adult neural circuitry (Saunders, 1966; Voyvodic, 1996). Hamburger and Oppenheim (1982) suggested "the *unifying principle* that competition for a trophic agent is the overriding factor in explaining natural neuronal death". Raff (1992) and Raff *et al.* (1993) broadened this concept to one of "social controls" of cell death, reviewing several forms of cell-cell interactions which determine neuronal death and survival. Other authors have stressed the importance of the timing of expression of growth factors in controlling culling (Cunningham *et al.*, 1981; Dreher and Robinson, 1988; Dreher *et al.*, 1996). Still other workers have stressed evidence of intrinsic time limits to the survival of developing neurones (Galli-Resta and Ensini, 1996; Galli-Resta and Resta, 1992).

A significant step in our recent work was the realisation that cell death occurs significantly later among photoreceptors than among other neurones of the retina and CNS (Section 2.1.2) and is regulated by different factors (Section 2.3).

2.1.2. *The culling of photoreceptors occurs late*

Prior to the development of the TUNEL technique (Gavrieli *et al.*, 1992) for the detection of dying cells *in situ* by the fragmentation of the nuclear DNA, dying cells were detected as pyknotic nuclei. Writing in 1991 Robinson, (1991, his Table 2.3), reviewed available data on retinal cell death from many authors for eight mammalian species. For three of these species cell death in the INL and ONL has been re-examined with the TUNEL technique (Maslim *et al.*, 1997). That re-examination confirmed the timing of cell death described previously for the INL, but suggested that cell death in the ONL lasts longer and peaks later than estimated from observation of pyknotic bodies. For the rat, ONL death appeared to peak at P16.5 when estimated from pyknotic bodies, but at P25 when estimated from TUNEL labelling (Fig. 1B). For the rabbit the corresponding

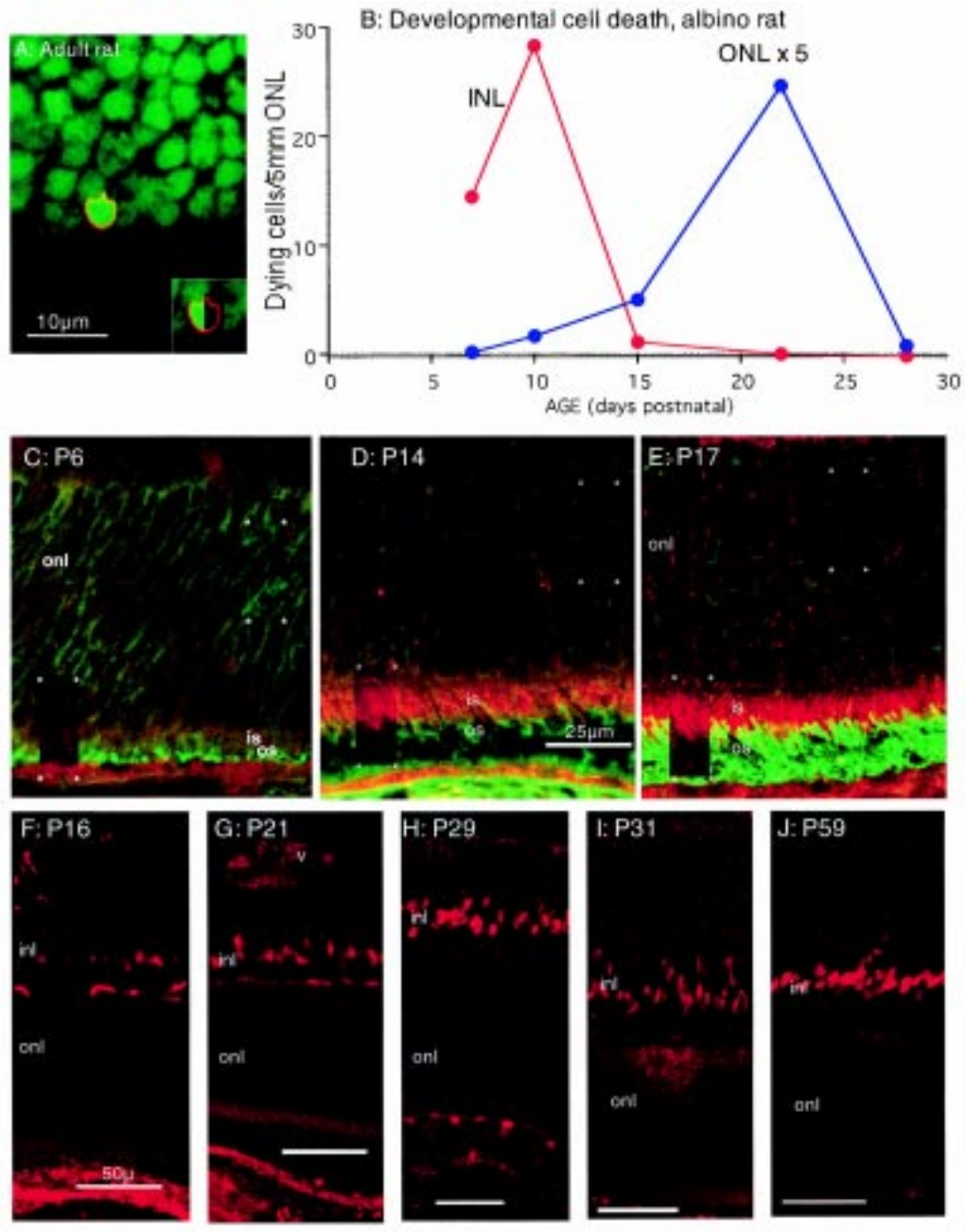

Fig. 1. (*Caption overleaf*)

estimates were P3 (from pyknosis) and P13 (TUNEL); and for the cat were P–5 (5d before birth) and P20. It also emerged that death among photoreceptors occurs later than among CNS neurones in general. In the rat for example, cell death in the inner layers of retina, which peaks at about P11 and is at very low levels by P13 (Maslim *et al.*, 1995, 1997; Fig. 1B), is contemporaneous with cell death in the neocortex, where death is largely complete by P12 (Ferrer *et al.*, 1990).

These later estimates of death among photoreceptors may be the more accurate because the amplification steps in the TUNEL technique make it more sensitive (Fig. 1A). If so, developmental death occurs significantly later among photoreceptors than among other retinal and CNS neurones, raising the possibility that it is determined by different factors. When we sought to identify those factors we were led to issues of photoreceptor metabolism.

2.2. Correlates of Photoreceptor Culling

2.2.1. *Morphology: growth of outer segments*

In the rat (the species for which data on photoreceptor culling are most detailed), the inner and outer segments of photoreceptors can be detected by light microscopy at about P7 and grow until P16 (Braekevelt and Hollenberg, 1970; Fig. 1C–E), thus approaching their adult length as naturally occurring cell death begins to become prominent (Fig. 1B). Similarly in the cat, inner and outer segments approach their adult length by P25 (Donovan, 1967), as developmental death of photoreceptors becomes prominent. In the rabbit, inner and outer segments grow between P2 and P17 (Reichenbach *et al.*, 1991), again preceding and during the onset of the period of developmental photoreceptor death.

2.2.2. *Function: development of the ERG*

The ERG, a gross measure of retinal function, develops as the inner and outer segments grow (reviewed in Maslim *et al.*, 1997), its amplitude in the cat and rat increasing towards adult levels before and then during the period of developmental photoreceptor death.

2.2.3. *Metabolism: sharp acceleration*

The onset of retinal function is accompanied by and presumably fuelled by a sharp acceleration in the glycolytic and oxidative metabolism of glucose (Fig. 2) (Graymore, 1959, 1960; Cohen and Noell, 1960). The developmental death of photoreceptors thus occurs shortly after the onset of their function and metabolism.

2.2.4. *Molecular correlate*

Many genes upregulate during the onset of the critical period, those relating to outer segment construction, photopigments and membrane

Fig. 1. Developmental death of photoreceptors. **A:** The outer part of the ONL of an adult rat labelled with a green fluorescent DNA-specific dye (SYTO 12, Molecular Probes) and with the TUNEL technique (red fluophore) for DNA fragmentation. In this field a single nucleus is TUNEL-labelled. The inset shows the labelled cell with the green DNA signal "blown" away in part, to show that the TUNEL labelling is limited to the external surface of the nucleus. This is considered an early stage in the apoptotic death, caused by activation of cytoplasmic nucleases (Gavrieli *et al.*, 1992). **B:** Developmental cell death in the INL and ONL of the non-dystrophic (albino) rat (from Maslim *et al.*, 1997). Death in the ONL begins, peaks and ends relatively later. The ONL counts have been multiplied by five, for purposes of presentation. **C–E**: Development of two key molecules in photoreceptor differentiation. An antibody to rod opsin (green, antibody generously provided by Dr. R. Molday) shows that between P6 and P17 the outer segments (os) of rods grow markedly. At the same time the concentration of opsin in somas in the ONL (seen in rectangular areas marked by asterisks in which the red fluophore has been deleted) reduces. Over the same period the concentration of cytochrome oxidase (red fluophore) grows in intensity, particularly in the inner segments (is) (seen in rectangular areas in which the green fluophore has been

channels. One protein particularly relevant to the onset of photoreceptor function is the enzyme cytochrome oxidase (CO). CO plays a key role in the oxidative metabolism of glucose and its concentration in the mitochondria of the inner segments of photoreceptors is particularly intense (Kageyama and Wong-Riley, 1984). As Fig. 1C–E shows, CO appears in the inner segments of rat photoreceptors between P8 and P17, coinciding with the onset of photoreceptor function and death (Bowers and Stone, 1998).

2.2.5. *Vasculogenesis: physiological hypoxia and hypoglycaemia*

In the rat the onset of photoreceptor metabolism induces a physiological episode of hypoxia in the middle and inner layers of retina (Stone *et al.*, 1995; Stone and Maslim, 1997). This episode induces retinal glial cells (astrocytes, Müller cells) to express the hypoxia-inducible (Shweiki *et al.*, 1992) angiogenic factor VEGF. Their expression of VEGF induces formation of vasculature which supplies oxygen to the retina, relieving the hypoxia and limiting the formation of new vessels. Hypoxia is thus an important and normal stimulus for some developmental events, and it was knowledge of this role of hypoxia as a developmental signal that led us to test whether hypoxia is a factor in regulating photoreceptor death (next Section).

VEGF expression can also be induced by hypoglycaemia (Stein *et al.*, 1995; Shweiki *et al.*, 1995) and it seems likely (though the point has yet to be tested) that the acceleration of photoreceptor metabolism which makes the retina hypoxic also makes it hypoglycaemic. Since glucose is the major source of retinal ATP, whether by glycolysis or the oxidative TCA cycle (Section 3.1.3), it will be important to test the role of hypoglycaemia in the regulation of photoreceptor death.

2.3. The Regulation of Culling

2.3.1. *Competition for oxygen, glucose*

Evidence that competition for oxygen is a factor in the developmental death among photo-

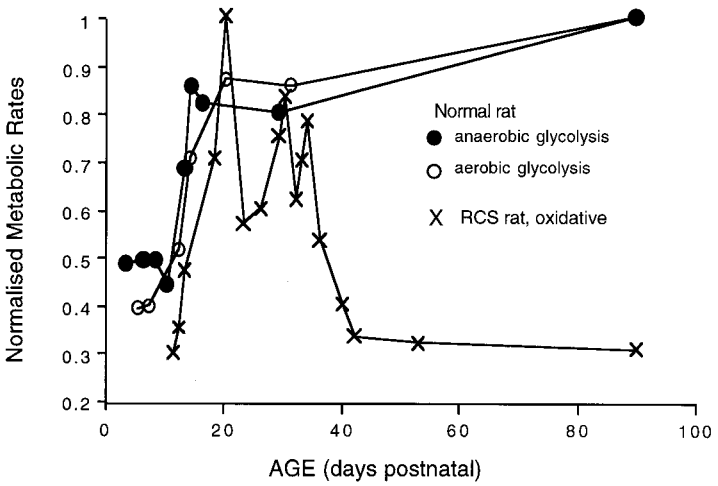

Fig. 2. Metabolic correlates of developmental photoreceptor death. Graymore (Graymore, 1959, 1960) quantified a rapid increase in the metabolism of the rat retina in early postnatal life. The increase was apparent as lactate/pyruvate production both in the absence of oxygen "anaerobic glycolysis" and in the presence of oxygen "aerobic glycolysis", and occurred between days 14 and 19, thus coinciding with the onset of developmental photoreceptor death (Fig. 1B). A simultaneous increase was detected in the rate of oxidative metabolism of glucose. The data shown here are for the RCS rat, and show a rise in oxidative metabolism between day 16 and day 20 followed by a fall caused by the dystrophy. This experiment was done to establish that the sharp acceleration of retinal metabolism occurs principally in the photoreceptors.

receptors of the rat was reported by Maslim *et al.* (1997) and Valter *et al.* (1998a,b). In brief, increasing the oxygen available to albino Sprague-Dawley (non-dystrophic) rat pups during the period of developmental death reduces the rate of death in a dose-related manner, while decreasing the oxygen available increases the rate of death markedly (Fig. 3B). Similarly, hyperoxia slows and hypoxia accelerates photoreceptor death in the dystrophic RCS rat. Further, Valter *et al.* (1998b) reported evidence that, at least in the RCS rat, these effects are not mediated by the neuroprotective factor bFGF (basic fibroblast growth factor); that is, hyperoxia (which slows developmental death of photoreceptors) did not cause an upregulation of bFGF. Mervin and Stone (1997) extended these observations to the non-dystrophic C57 black mouse. Hyperoxia during the period of developmental cell death (approximately P8–16) slowed and hypoxia greatly increased the rate of photoreceptor death (Fig. 3A).

As the period of developmental photoreceptor death comes to an end, between P25 and P30 in the rat, the rescue effect of hyperoxia can no longer be tested. The efficacy of hypoxia in inducing photoreceptor death has been tested in older mice and rats (Mervin and Stone, 1997; Valter *et al.*, 1998; Fig. 3). In both species hypoxia kills adult photoreceptor cells but in much smaller numbers than in the juvenile.

There are no published data on whether blood glucose levels influence developmental photoreceptor death. Because glucose is the major dietary substrate for ATP generation in the retina (whether by oxidative or glycolytic metabolism),

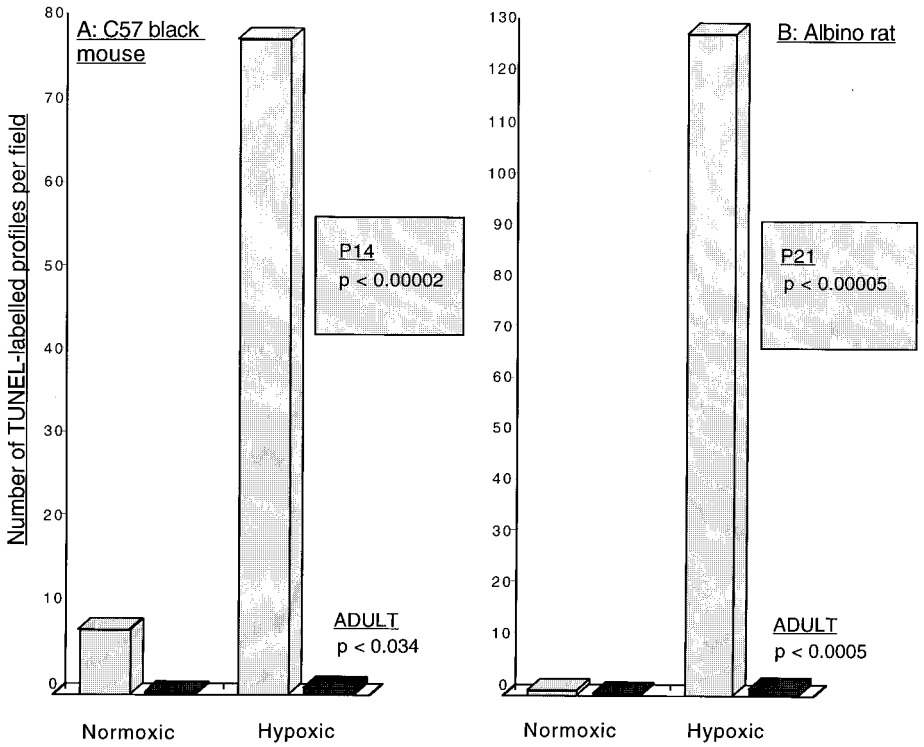

Fig. 3. Response of photoreceptors in adult and juvenile retina to hypoxia. In both the pigmented C57 black mouse (A) and the albino rat (B), hypoxia (breathing air containing 10% oxygen) accelerated the rate of death among photoreceptors. The adults were kept in hypoxia for up to 7 days, the juveniles from P8–P15 (mouse) or from P15–24 (rat). The increase was much greater in juvenile animals than in adults. The smaller response in adults was statistically significant in both species.

the potential for blood glucose levels to play a role in photoreceptor culling seems high.

2.3.2. *Culling by energy competition is specific to photoreceptors*

Two observations suggest that competition for energy is not important in the culling of other retinal neurones, at least during the period when that competition is important for photoreceptors. Maslim *et al.* (1997) tested whether hyperoxia could slow developmental cell death in the INL, and reported no influence. Conversely the acceleration of cell death caused by hypoxia in their experiments was confined almost completely to photoreceptors. Neurones of the inner nuclear layer and ganglion cell layers were little affected, if at all.

2.3.3. *What ends the lethality of competition?*

The death of photoreceptors during normal development is thus regulated at least in part by metabolic stress and their death slows and then ends, in part, because the death of some photoreceptors reduces metabolic stress on the survivors. The competition among photoreceptors for energy presumably extends into adult life however. The outer layers of retina become hypoxic each night, for example, as the metabolism of rods increases when they dark adapt (Linsenmeier, 1986). This nightly episode of hypoxia is not known to induce photoreceptor death and, even when the severity of hypoxia of adult retina is experimentally increased, the photoreceptor death which occurs is much less than in the juvenile (Fig. 3). What has changed?

Because of evidence (Section 3.2) that the retina can express cytokines with protective properties, such as bFGF, we examined the distribution of bFGF protein in the retina during the period of developmental photoreceptor death. Early in this period bFGF protein is detectable in the somas of Müller cells and in blood vessels (Fig. 1F) and in the RPE (apparent in Fig. 1H), and the level of bFGF in these structures seems to increase to P29 (Fig. 1F–H). Among neurones, bFGF was detectable in the somas of ganglion cells and the inner segments of photoreceptors, but at relatively low levels (not shown). At P31 and older (Fig. 1I,J) bFGF protein became detectable, and in specific regions of the retina became prominent in the somas of photoreceptors. We argue in Section 3.2 and Section 3.3 that the appearance of bFGF in photoreceptor somas is induced by stress (Fig. 4) and is associated with photoreceptor resistance to stress. Cao *et al.* (1997a) reported that the ability of the rat retina to upregulate its expression of bFGF in response to damage is minimal as late as P22 (well into the critical period) and reaches adult levels at about P90, well after the period of developmental photoreceptor death. It seems possible that the appearance of bFGF in photoreceptor somas at about P30 is a factor in ending the period of their culling.

A feature of the development sequence in Fig. 1F–J is that bFGF does not appear in photoreceptor somas until well after it has appeared in other retinal neurones, in the RPE and in blood vessels. It seems possible that the expression of bFGF, and perhaps of other protective cytokines in photoreceptors is delayed *in order to create* a period of vulnerability.

2.4. The Critical Period: Programmed Vulnerability

2.4.1. *The purpose of vulnerability*

The idea just suggested, that the end of the period of developmental death among photoreceptors is ''programmed'' as a delay in the expression of protective cytokines in photoreceptors, led us to speculate that the period has a teleological purpose. Given program and purpose, it would be appropriate to term the period a ''critical period''.

2.4.1.1. *Matching energy demand to supply by lethal competition*

One possible purpose of a critical period of photoreceptor vulnerability to metabolic stress is to match the population of photoreceptors to the supply of metabolic substrates (glucose, oxygen). The retina (we suggest) samples the levels of substrates available in early postnatal life, adjusts the

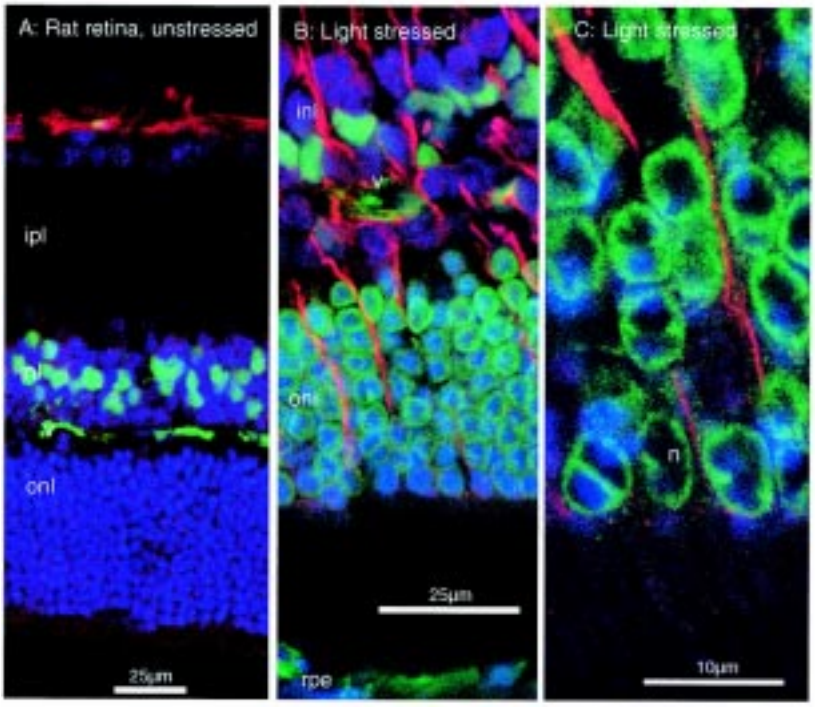

Fig. 4. Effect of bright light stress on bFGF protein expression in adult photoreceptors. **A:** In adult rat retina unstressed by exposure to bright continuous light (BCL) bFGF (green) is prominent in Müller cell somas in the INL, in the walls of vessels (v) and in the nuclei of astrocytes in the axon layer. Astrocytes are labelled red by an antibody to GFAP; one astrocyte nucleus can be seen detected as a yellow spot among the astrocytes. The blue label is a DNA dye. The dye used (TOTO3, Molecular Probes) works in the infrared range and is placed by software in the blue channel of these images. **B:** After 24 h exposure to BCL, two signs of stress are apparent. Processes of Müller cells are labelled for GFAP (red) and bFGF protein (green) is prominent not only in vessels (v) but also in the somas of photoreceptors in the ONL. **C:** bFGF seems localised to the cytoplasm of the somas. The nuclei of these cells (n) are free of label.

photoreceptor population to that availability and then, assuming that any future interruption to that supply will be temporary, makes the survivors resistant to metabolic stress.

2.4.1.2. *Energy matching after the critical period*

Whatever the purpose of the period of photoreceptor vulnerability, its end marks a strategic shift in the response of the retina to a deficit in energy supply. During the period, the retina's response is to reduce the photoreceptor population towards the energy available. After the critical period the photoreceptors respond by survival mechanisms, such as the switching of energy sources (Section 3.1) or the upregulation of protective cytokines (Section 3.3.4, Section 3.4.1), if necessary at the cost of retinal sensitivity (Section 3.4). Photoreceptors then survive energy starvation except in quite extreme conditions, such as retinal detachment (Section 4.1).

2.4.2. *Dangers of vulnerability: photoreceptor depletion*

Episodes of stress during the critical period might be expected to cause unusual levels of photoreceptor death which (it is proposed in Section 5 below) can precipitate total destruction of the photoreceptor population. Conversely it might be expected that many photoreceptor dystrophies will commence during the critical period.

2.4.2.1. *Genetic dystrophies begin with the critical period*

The time of commencement of human dystrophies is not well established (Section 6.2.1). Of

the dystrophies much studied in rodents, however, many start in the critical period. In the rat, for example, the autosomal recessive RCS dystrophy begins at the same time as the period of developmental cell death in this species (about P15, Maslim *et al.*, 1997, Fig. 5B), then accelerates and proceeds to exhaustion. In dystrophies created by implantation into rats of opsin mutations which generate RP in humans (the P23H and S334ter mutations) the dystrophy also begins in the critical period, and in some strains is near-complete by P30 (Nishikawa *et al.*, 1997). In the *rd* mouse, in which a rod-cone dystrophy is precipitated by an autosomal recessive mutation of an enzyme important in the phototransduction cascade (cGMP phosphodiesterase, Farber *et al.*, 1994; Farber, 1995), the degeneration of rods again begins early (at about P8, Chang *et al.*, 1993; Lolley *et al.*, 1994), coinciding with the onset of the critical period in the genetically normal C57 mouse (Mervin and Stone, 1998; Fig. 5A).

The *rds* mouse is an interesting exception to this rule. The "s" in *rds* stands for "slow"; the degeneration is so termed because it begins later and occurs more slowly than, for example, the *rd* dystrophy. The excess degeneration of photoreceptors is not apparent until P21 (Chang *et al.*, 1993), thus after the critical period of non-dystrophic mice. Arguably, the *rds* exception confirms the rule. In the homozygous *rds* mouse the outer segments of photoreceptors do not develop beyond rudiments (Travis *et al.*, 1992). The sharp increase in retinal metabolism which normally

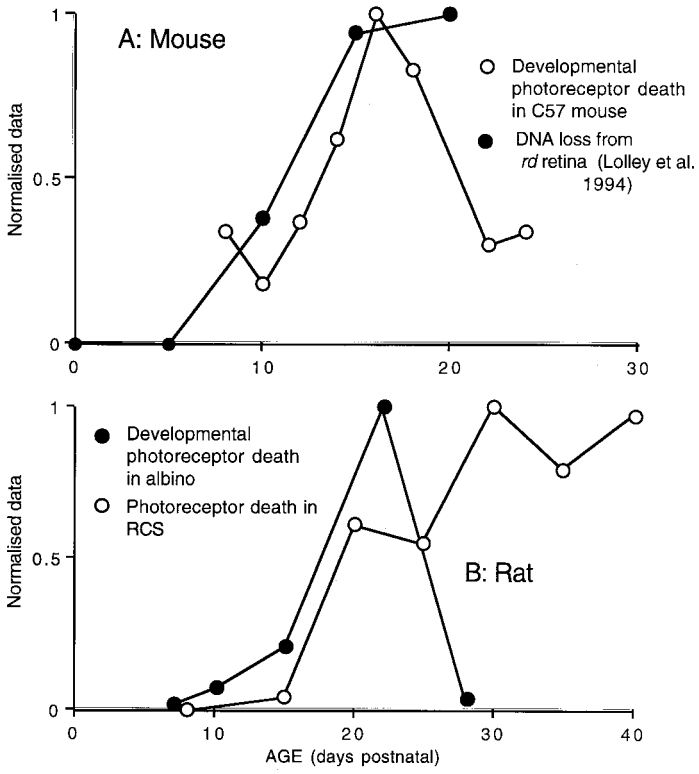

Fig. 5. When dystrophies begin. **A:** Data are shown for the frequency of TUNEL-labelled (dying) photoreceptors in the normally developing retina of the C57 black mouse, and for the loss of DNA during the *rd* dystrophy, using data from Lolley *et al.* (1994). **B:** Data are shown for the frequency of dying photoreceptors in the normally developing retina of the non-dystrophic albino rat, and for the dystrophic RCS rat. For purposes of comparison, all values are normalised to their maximum in the data sets used. The point of these graphs is the evidence they provide that the dystrophies begin at the time of naturally occurring photoreceptor death.

accompanies the growth and onset of function in outer segments (Section 2.2.3) presumably does not occur in this strain, so that the critical period does not begin. Travis *et al.* (1991) suggested that the slow degeneration which eventually destroys the photoreceptors of this strain may result from a quite different cause, oxygen toxicity (Section 4.2).

2.4.2.2. *Genetic and environmental factors can summate*

In the RCS rat the dystrophy of photoreceptors is caused by a mutation which disturbs the normal process by which the RPE phagocytoses the discarded tips of photoreceptors (Li and Turner, 1988; LaVail *et al.*, 1992a). The dystrophy is also caused by hypoxia (Valter *et al.*, 1998). These two lines of evidence are not contradictory, for there is evidence (Valter *et al.*, 1998) that the failure of phagocytosis by the RPE causes an accumulation of debris in the subretinal space of this strain, and that the debris blocks the flow of oxygen from the choroid to the photoreceptors, creating a severe hypoxia during the critical period. In short, genetic and environmental factors combine in this strain to drive the initial stages of the dystrophy. It is possible that environmental factors play a role in genesis of other genetically precipitated dystrophies, including those seen in humans.

2.5. The Critical Period in Humans

In rodents the critical period of vulnerability has morphological correlates which can be checked in human material. In the human the retina develops over a much longer period than in the rat, and with a longer centro-peripheral delay of weeks instead of days (Provis *et al.*, 1985). Nevertheless, except for the specialised events related to the formation of the fovea, the major events of retinal development occur in the human in a sequence common to all mammals (Robinson, 1991).

The population of photoreceptors differentiates over a prolonged period, cones differentiating before rods. The rods become evident on the rim of the incipient fovea by week 13 (Diaz-Araya and Provis, 1992) but do not become evident in peripheral retina until as late as week 30. The timing of the development of rod outer segments in human retina has not been studied in detail but labelling for rod opsin indicates that rod outer segments in the central area are relatively undeveloped at week 35 (Fig. 6A). A wave of cell death affects the INL between week 15 and about week 30, and by week 35 only low levels of cell death are seen in the INL or ONL, in either central or peripheral retina (Fig. 6B,C). A similar pattern of cell death is seen in animal models, such as the rat and cat before the period of developmental photoreceptor death (Section 2.1). At week 41, in peripheral retina, the inner and outer segments have begun to grow but are short and incompletely differentiated (Fig. 6D) and cell death is low in both the INL and ONL (Fig. 6F,H). However, in the more central (developmentally more advanced) regions of the retina at this age, the inner and outer segments of rod photoreceptors are longer and more differentiated (Fig. 6E); cell death is virtually absent from the INL but evident in the ONL (Fig. 6G,I). This pattern suggests that by week 41 a period of naturally occurring photoreceptor death, comparable to that observed in rodents, has begun in the central region. Material is not available to enable identification of the end of this period.

In rodents, the period of naturally occurring cell death corresponds approximately to the critical period, raising the possibility that in humans the critical period begins in the central retina between gestational weeks 35 and 41 and extends for a still undefined period. Since humans are born at about week 40 it is possible that the critical period of human photoreceptor development is "on" at the time of birth and persists into early neonatal life.

3. MECHANISMS OF ADULT STABILITY: ADAPTABILITY AND SELF-PROTECTION:

3.1. Adaptability of Energy Sourcing

"The...retina has three unusual properties: it converts glucose to lactic acid with prodigious speed; it consumes oxygen more

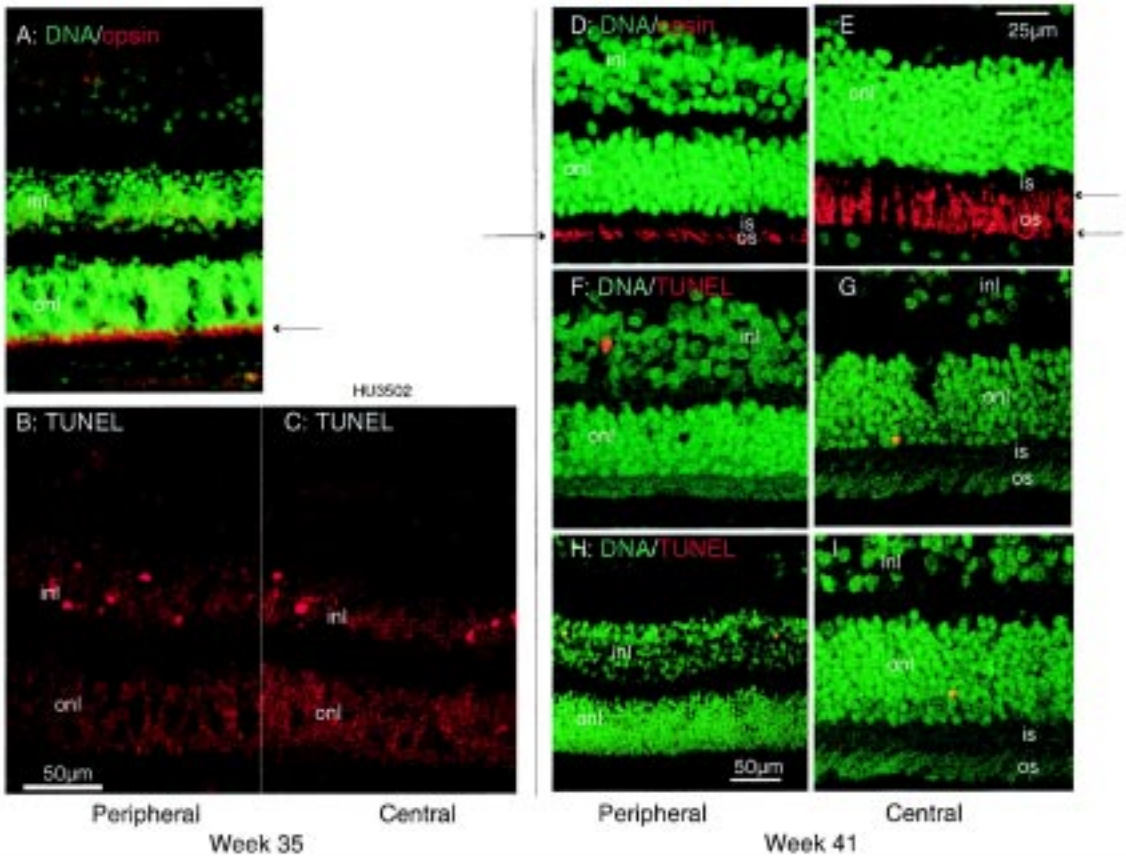

Fig. 6. Evidence of developmental photoreceptor death in humans. Images are from human retinas aged 35 (A–C) and 41 weeks post-conception (D–I). At week 35 the inner and outer nuclear layers (inl, onl) are well separated but the outer segments (labelled red with an antibody to rod opsin) are very short (arrowed in A). At this age, cell death (red, TUNEL) is prominent in the INL but not the ONL (B, C). At week 41, the outer segments (labelled red with the antibody to opsin in D and E) are longer than at week 35, even in peripheral retina (arrow in D) and more markedly so in central retina (arrows in E). The inner segments, or at least their location (is), are now apparent. At this age, cell death (red in F–I)) is persisting in the INL in peripheral retina (F, H). In central retina, however, death in the INL is close to zero, and some photoreceptors are dying (G, H). The scale in B refers to A, B, C. The scale in E refers to D, E, F, G, I.

readily than other tissues; and the formation of lactic acid is rapid even in the presence of oxygen....'' Cohen and Noell (1965)

3.1.1. *The high aerobic metabolism of photoreceptors*

The central nervous system (the retina included) uses ATP as its energy currency and generates that ATP from glucose, rather than from protein or fats (Winkler, 1995). The retina metabolises glucose to pyruvate, producing 2M ATP per mole glucose in a process termed glycolysis. The pyruvate is then either converted to lactate and removed by the circulation or oxidised in the TCA cycle, producing another 36 mole ATP per mole of glucose (Winkler, 1995). Work with oxygen microelectrodes has provided evidence that photoreceptors use 3–4 times more oxygen than other retinal and CNS neurones (Alder *et al*., 1990; Braun *et al*., 1995) and twice as much again in dark adapted conditions (Linsenmeier, 1986; Brown *et al*., 1996) and are probably the cells of the body with the highest rate of oxidative metabolism. The site of this very high oxidative metabolism is the concentration of mitochondria in the inner segments. The ATP produced there goes to meet the very high energy cost

of maintaining the structure and ionic polarisation of the outer segment (Ames *et al.*, 1992; Demontis *et al.*, 1997), particularly of Na^+ transport.

3.1.2. *The high anaerobic capacity of the retina*

Despite the high rate of oxidative metabolism of glucose in photoreceptors, most (90%, Winkler, 1995; >80%, Wang *et al.*, 1997) of the glucose utilised by photoreceptors is not oxidised, but is metabolised to pyruvate, which is then converted to lactate and leaves the tissue by entering the bloodstream. This non-oxidative metabolism of glucose to pyruvate is termed glycolysis. Because glycolysis is so much less efficient than oxidative metabolism, this 90% of glucose metabolised glycolytically produces about 33% of the ATP used by photoreceptors, while the 10% of glucose metabolised oxidatively produces 66% of the ATP (Winkler, 1995).

The experiments which established the retina's high rate of aerobic glycolysis were done on the whole retina, and could not identify which cell classes are responsible. By comparing the metabolism of the normal retina with that of retinas depleted of photoreceptors by the RCS dystrophy (Graymore, 1960) or by iodoacetate poisoning (Graymore and Tansley, 1959), evidence was gained (Fig. 2) that the high metabolic capacities of the retina—both oxidative and glycolytic—lie in the photoreceptors.

3.1.3. *Photoreceptors can switch energy sources*

The photoreceptors' high requirement for ATP, and their high rates of oxidative and anaerobic metabolism to produce that ATP, could make them doubly vulnerable, to both hypoxia and hypoglycaemia. Instead the photoreceptors have evolved a protective ability to upregulate aerobic metabolism in the face of hypoglycaemia and anaerobic metabolism in the face of hypoxia.

One early measure of the retina's ability to switch energy sources came from wartime work of McFarland and colleagues (McFarland and Forbes, 1940; McFarland *et al.*, 1945) on vision in dark-adapted hypoxic conditions which mimicked those experienced by pilots at night. They showed that lowering atmospheric oxygen tension causes a rise in dark-adapted thresholds (explaining the dimming of vision at altitude reported by pilots). They went on to show that the threshold rise induced by hypoxia was swiftly reversed by providing the subjects with either more oxygen (restoring oxygen levels to normal) or more glucose (80 gm by mouth, probably—since the subjects were air force recruits—a candy bar).

At the cellular level, Fig. 7 shows one of many approaches used by Winkler (1995) to assess the interaction between glycolytic and oxidative production of ATP in the whole retina. In the absence of glucose and oxygen, the retina can produce little ATP. With glucose plentiful (20 mM) however, ATP production was high, reaching 75% of control levels even in the complete absence of oxygen. Conversely, with glucose levels 20 times lower (1 mM) but in normal levels of oxygen, ATP production reached 85% of control levels by the efficient oxidative metabolism of the limited glucose available. So, if pushed, the retina can produce 75% of the ATP it needs by glycolysis and 85% by oxidative metabolism; when both oxygen and glucose are available, the retina has excess capacity for ATP production. At the functional level, Winkler (1983, 1995), showed in a range of tests of the ERG that the response of photoreceptors (the a-wave) is more durable than the response of inner retina (the b-wave) in the face of both hypoxia and hypoglycaemia. Kang and Linsenmeier (1998) have recently confirmed the robustness of the a-wave in the face of hypoxia.

Overall, the energy adaptability of the photoreceptor is a strongly protective adaptation to the risks of starvation posed by its high appetite.

3.2. Physiological Stresses Induce Expression of Protective Cytokines

In the studies which established the ability of neurotrophic factors to protect photoreceptors from genetic and light damage (Faktorovich *et al.*, 1990, 1992; LaVail *et al.*, 1992b) candidate factors were selected by their ability to protect

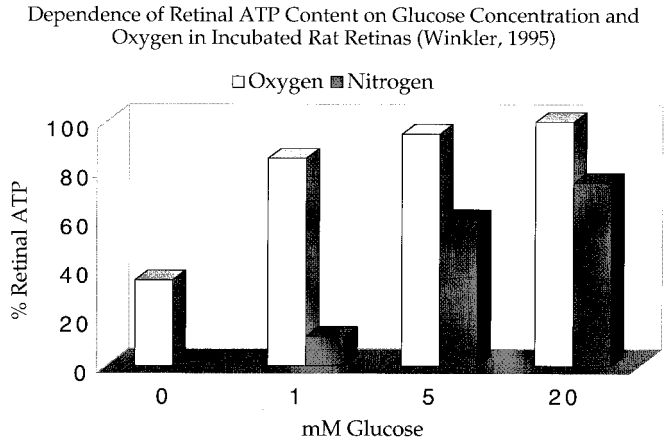

Fig. 7. The ability of the retina to switch energy sources. Data, redrawn from Figure 3 of Winkler (1995) show the ability of the isolated retina to produce ATP (normalised) in two conditions of oxygen availability (100% nitrogen or 100% oxygen in atmosphere over the medium) and four levels of glucose availability (mM in the incubation medium). The data show (see text) that in the absence of oxygen, the retina can produce 75% of its ATP needs by glycolysis; and that in the presence of minimal (1 mM) glucose and 100% oxygen, the retina can produce 85% of its ATP needs. The totals of these two capacities (160%) is a measure of the capacity of the retina to switch between glycolysis and oxidative metabolism of glucose.

neurones in other systems. They were introduced as factors exogenous to the retina but with therapeutic potential. It subsequently became clear, however, that the retina can express protective factors endogenously, that it does so in response to damaging stimuli and that endogenously expressed factors are strongly protective to photoreceptors. It has emerged moreover that these mechanisms function in physiological as well as pathological conditions, that the genetically normal retina experiencing natural levels of daylight in a normal day/night cycle continuously uses these factors to condition itself by recent experience for the stresses of the future.

3.2.1. *Normal light experience induces cytokine and antioxidant expression*

3.2.1.1. *Circadian light, damage and survival*

Penn and colleagues (Penn *et al.*, 1987; Penn and Anderson, 1987; Penn *et al.*, 1989: reviewed in Penn and Anderson, 1991), developed two major insights into environmental influences on the death and survival of photoreceptors. First, they showed that the genetically normal retina is damaged by normal light experience. They raised genetically normal rats in cyclic light in three groups, with the brightness of the light phase set at one of three levels: dim (5 lux), bright (300 lux) or very bright (800 lux). At the age of 3 months, all animals had functional, intact retinas but the retinas of rats raised in brighter light had fewer photoreceptors, with shorter outer segments, more damaged membranes and less rhodopsin than those raised in dim light and correspondingly yielded a weaker ERG. This observation suggests that light-induced damage in photoreceptors is as normal and adaptive as tread-induced roughening of the skin on the sole of the foot.

Their second finding was a paradox rich in implication. When exposed to potentially damaging light, the highly intact dim-raised retinas were devastated, while retinas that were depleted and scarred because they had been raised in bright light emerged unscathed. Thus the retina uses damaging experience to upregulate self-protective mechanisms. Without the experience of bright light, the retina is beautifully intact, highly sensitive and defenceless.

3.2.1.2. *Mechanisms of light-induced protection: antioxidants and trophic factors*

Two responses have been identified in the retina which are light-induced and protective. Penn

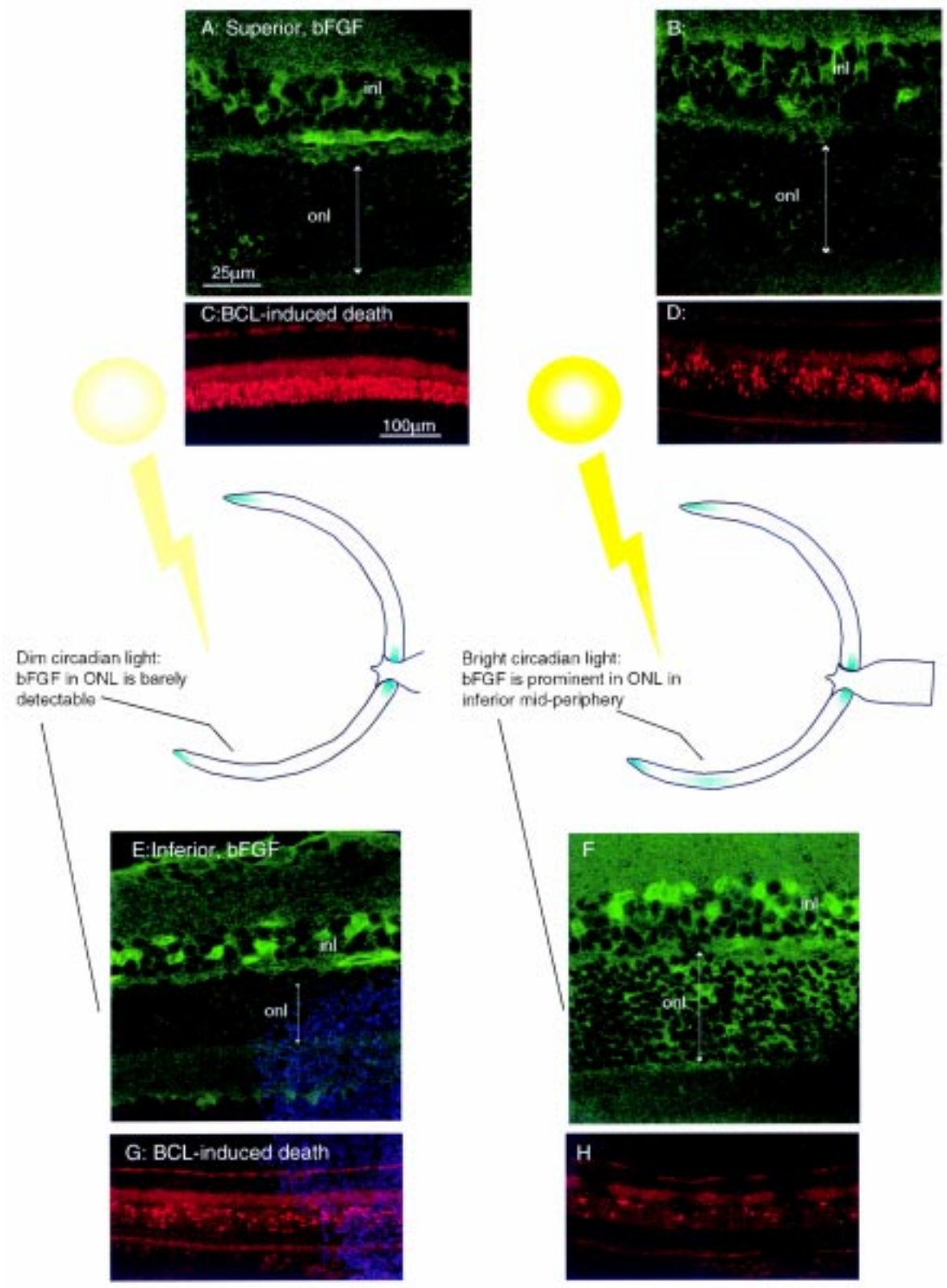

Fig. 8. (*Caption opposite*)

et al. (1987) tested antioxidant levels in the retinas of three groups of rats (dim-, bright-, very bright-reared) described in the previous Section. The levels of all antioxidants tested (vitamin E, ascorbic acid, glutathione) were higher in the retinas raised in the brighter light conditions. This implies that the damage caused by bright circadian light is caused at least partly by an oxygen toxicity, perhaps (Section 6.3.1.3) by the daily rise in oxygen tension of the outer retina caused by the light adaptation of rods (Linsenmeier, 1986; Brown *et al.*, 1996). In confirmation, Organisciak *et al.* (1998a) have recently shown the effectiveness of an antioxidant delivered systemically in limiting light-induced damage to the retina.

Second, Liu *et al.* (1998) showed that conditioning the retina with an episode of very bright light protects the retina against subsequent challenge by the same light, and that the rescue effect is associated with an upregulation of expression of two trophic factors, bFGF and CNTF (ciliary neurotrophic factor). We noted in our experiments that in rats raised in physiological levels (40 lux) of circadian (12 h on/12 h off) light, bFGF levels in the ONL are higher in inferior retina than superior retina (Fig. 8A,B) (probably because the light sources were above the rearing cage, see next Section) and that photoreceptor death induced by a very bright, continuous light was less in inferior retina (Fig. 8C,D). We next repeated Penn and Anderson (1991) model, conditioning albino rats for at least three weeks in circadian (12 h on, 12 h darkness) light of two brightnesses (200 lux and 5 lux). The brighter circadian light induced a higher concentration of bFGF in photoreceptors in inferior retina (Fig. 8D,E). We then challenged rats from these two groups with very bright (1400 lux) continuous (48 h) light and confirmed Penn and Anderson's (1991) observation that the retina conditioned to brighter circadian light suffered less damage (Fig. 8G,H).

Thus, both brief exposure to intensities capable of destroying the retina (Liu *et al.*, 1998) and longer-term physiological, circadian exposure induce bFGF concentration in the retina, and in photoreceptors in particular. However induced, the concentration of bFGF (and presumably of other factors) appears to be protective.

3.2.2. *Skylight and sidelight: cytokine protection is a local retinal response*

The relative vulnerability of superior retina to light damage described in the previous Section had been reported previously (Duncan and O'Steen, 1986). At least in our experiments the rats had been raised in rooms where the lights were located in the ceiling and it seemed possible that the inferior retina contained more bFGF (Fig. 8) and was more resistant because it had been more exposed to the conditioning light. To test the point we placed adult rats which had been initially raised in rooms with ceiling lights in boxes with black ceilings and a light source to one side. Over three weeks the concentration of bFGF in the ONL in the inferior retina faded, and a new concentration appeared in the superior retina 1–3 mm above the optic disc (Fig. 9B, lower diagrams). Further, when tested with the same damaging light stimulus which produced the extensive degeneration of superior retina shown in Fig. 8C, retinas conditioned to "sidelight" showed almost no degeneration at all in the superior retina (Fig. 9C). Sidelight conditioning had ended the vulnerability of superior retina.

These results suggest that the retina continuously regulates the levels of protective cytokines such as bFGF in the ONL at a local level and

Fig. 8. Effect of normal light rearing on retinal bFGF levels and vulnerability. Rats were raised conventionally, in a 12 h/12 h light dark cycle, with the lights in the ceiling. The lights were either 200 lux (bright) or 5 lux (dim). In the bright reared animals bFGF protein levels were higher in both inner and outer nuclear layers in the inferior mid-peripheral retina than in superior retina (compare B and F). A similar but weaker difference was apparent in the dim-raised retinas (compare A and E, inner nuclear layer); importantly, bFGF levels in inferior retina were lower in the dim-raised animals (compare E and F). The double headed arrows mark the width of the ONL. *Onl* designates the ONL, *inl* the inner nuclear layer. When littermates similarly raised were exposed to 48 h BCL, photoreceptors were induced to die, and became $TUNEL^+$ (C, D, G, H). Death rates were less in the bright reared animals than in dim-reared, and less in inferior retina than in superior.

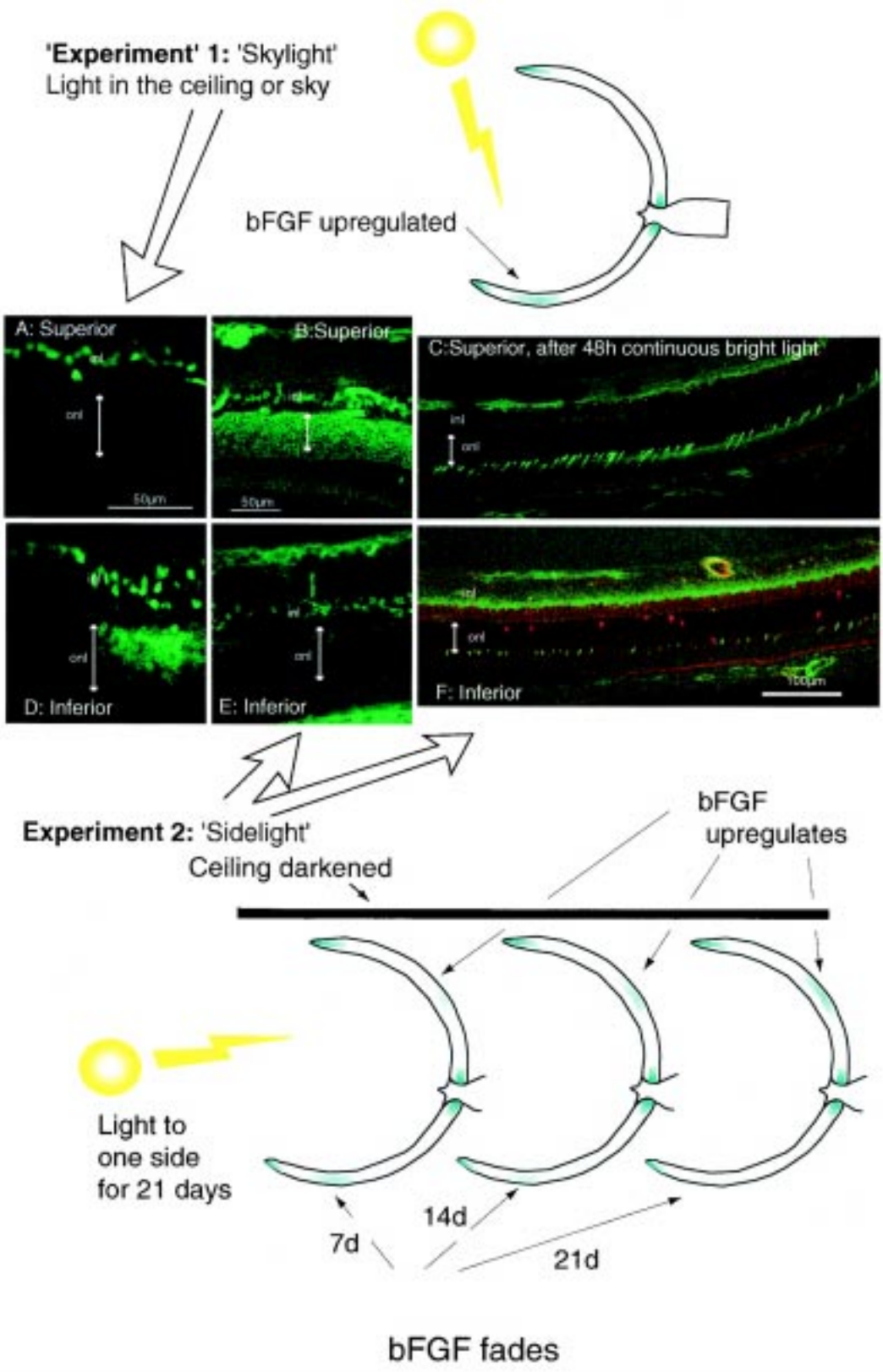

Fig. 9. (*Caption opposite*)

does so on a continuing basis, in response to day-to-day (or perhaps week-to-week) variations in light experience. The slow time course of bFGF regulation suggests the function of the continuous regulation of bFGF is prospective, using current experience to protect against future exposure.

3.2.3. *Surviving at the edge: a ring of protection?*

The retina has two edges, its peripheral anterior edge and a much shorter edge around the optic disc. It is a consistent feature of adult rat retina that bFGF is strongly upregulated in the ONL at both edges (confirming Xiao *et al.*, 1998) and that the protein GFAP is upregulated in Müller cells at the same sites (Fig. 10). Upregulation of GFAP in Müller cells is a sign of environmental (Eisenfeld *et al.*, 1984; Burns and Robles, 1990; Raad *et al.*, 1996), genetic (Eisenfeld *et al.*, 1984; Ekström *et al.*, 1988) or traumatic (Björklund *et al.*, 1985; Erickson *et al.*, 1987; Humphrey *et al.*, 1993a; Cao *et al.*, 1997a) stress to the retina, and a comparable upregulation of GFAP in astrocytes of the brain has been reported in hypoxia-stressed brain (Zimmer *et al.*, 1991). Upregulation of bFGF in the ONL is also a feature of normal mouse retina (Fig. 10C) and of normal human retina (Li *et al.*, 1997).

The stress which induces upregulation of bFGF and GFAP at the edges of the retina has not been identified. One possibility is that the edges of the retina are exposed to relatively high oxygen tension. Oxygen reaches all areas of the retina by diffusion across the RPE from the choroid. This flow may be supplemented at the peripheral edge by flow of oxygen "around the edge" (Valter *et al.*, 1998) or, at the optic disc, by oxygen flowing from the large vessels which traverse the nerve head. Whatever its cause, the concentration of bFGF in photoreceptors at the edges of the retina is much greater than the concentrations induced by circadian light in mid-peripheral retina and is presumably one factor making photoreceptors at the edges resistant to damage from hypoxia (Valter *et al.*, 1998), from bright continuous light (Bowers *et al.*, 1998b), from the oxygen toxicity which follows light damage (Figs 15 and 16) and from the late stages of the RCS dystrophy (LaVail and Battelle, 1975). It may prove useful to view the "rings" of high bFGF levels in the ONL around each of the two edges of the retina as barriers against external stress.

3.2.4. *How many factors?*

The work of LaVail and colleagues (LaVail *et al.*, 1992b) emphasised how many cytokines can rescue photoreceptors when applied exogenously (by intravitreal injection). Several studies (Kostyk *et al.*, 1994; Wen *et al.*, 1995; Wen *et al.*, 1996; Cao *et al.*, 1997b; Liu *et al.*, 1998) have shown that the retina expresses two of these factors (bFGF and CNTF) endogenously in response to stimuli which induce retinal self-protection and in spatial and temporal patterns which correlate with protection. Conversely, Campochiaro *et al.* (1996) showed that in the retinas of transgenic mice with mutant receptors for bFGF the photoreceptors undergo a slow but eventually devastating degeneration. The evidence that at least CNTF and bFGF are self-protective factors endogenous to the retina seems compelling.

Others, using different tools, have noted that photoreceptor rescue correlates with the upregulation of quite different genes, the heat shock proteins (Section 3.3.3) and endogenous antioxidant

Fig. 9. Cytokine-mediated protection is continuously adjusted by the retina on a local basis. In rats raised in "skylight" (lights in the ceiling) of conventional brightness (200 lux) and period (12 h on, 12 h off) bFGF protein levels in the ONL are higher in inferior retina than superior (compare A and D, confirming Fig. 8). The double-headed arrows mark the width of the ONL. When rats raised in skylight were raised for a further three weeks in "sidelight", with the ceiling darkened and the light at the height of the cage floor, on one side of the cage, the prior concentration of bFGF in the inferior mid-periphery of the retina faded and a new concentration appeared in superior retina (B and E). The fading and the new appearance of bFGF extended over approximately three weeks. When rats raised first in skylight and then in sidelight for three weeks were exposed to BCL (parameters as in Fig. 8) with the light source above the cage, the superior retina proved highly resistant (compare Fig. 9C with Fig. 8C). Figure 9C and D are labelled with the TUNEL reaction (red) and with a PNA lectin which shows cone sheaths (green).

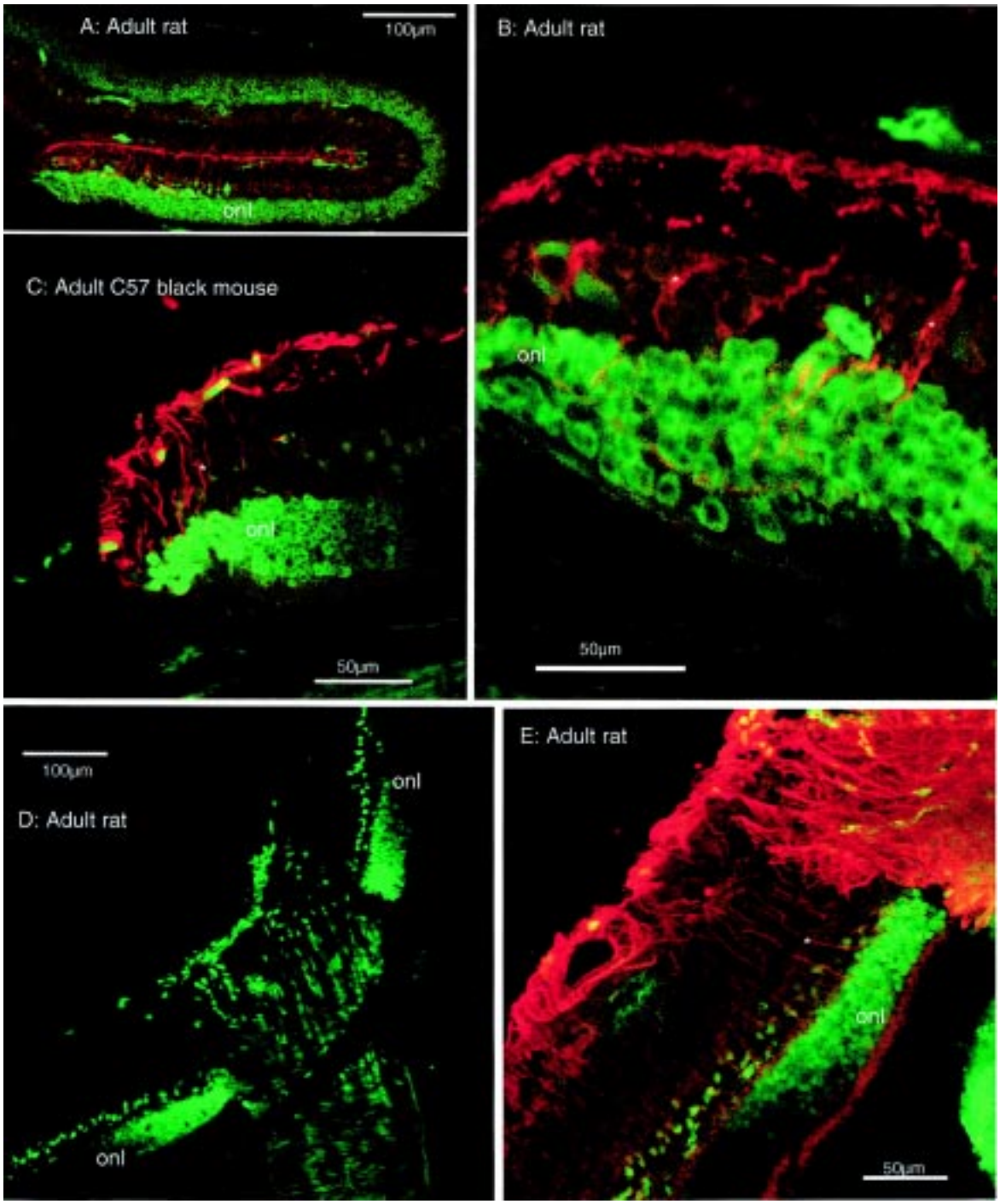

Fig. 10. (*Caption opposite*)

mechanisms (Section 3.2.1.2). Self-protection seems to involve several mechanisms and more than several factors.

3.3. Pathological Stresses Induce Expression of Protective Cytokines

3.3.1. *Continuous light*

Continuous light damages the photoreceptors of the rat's retina (Noell *et al.*, 1966). This damage is associated with an increase in the levels of bFGF protein in photoreceptors (Fig. 11B,D,F). The strong increases seen in Fig. 11B,D seem to be an exaggerated form of the relationship noted above (Section 3.2.1.2) between the daily light experience of the retina and the levels of bFGF protein in the ONL. Liu *et al.* (1998) showed that a 12 h exposure to bright light, which if continued caused major retinal damage, caused minimal photoreceptor death but upregulated both bFGF and CNTF (though their data did not indicate in which layers), and made the retina resistant to subsequent challenge by the same light.

3.3.2. *Incision and laser burn*

Trauma to a patch of retina makes photoreceptors around the wound resistant to damage. This was shown by Faktorovich *et al.* (1992) in control observations for their study of the protective effects of growth factors injected into the eye and by Behbehani *et al.* (1984) and Humphrey *et al.* (1993b) who studied the effect of small laser lesions on the viability of surrounding retina in the dystrophic RCS rat. Both needlestick and laser injuries upregulate the expression of trophic factors in photoreceptors near the wound (Wen *et al.*, 1995; Xiao *et al.*, 1998; Chu *et al.*, 1998) suggesting that the protection of surrounding photoreceptors is mediated by these factors.

3.3.3. *Heat*

Barbe *et al.* (1988) reported that stressing the retina with heat (41°C for 15 min) caused the expression of heat shock proteins (HSPs) in the retina. If the retina was challenged with potentially damaging light 4 h after heat stress, when HSP synthesis was maximal, the damage caused was less than in retinas not prestressed by heat. Hyperthermia coincident with damaging light exacerbated the damage caused (Organisciak *et al.*, 1995), suggesting that heat *per se* is damaging to the retina. Barbe *et al.* suggest that the HSPs upregulated by the heat stress are a mechanism of retinal self-protection.

3.3.4. *Hypoxia*

We have reported (Valter *et al.*, 1998) that levels of hypoxia which increase the rate of photoreceptor death in the RCS rat also increase the levels of bFGF protein in the retina and of bFGF mRNA in the ONL.

3.3.5. *Genetic stress—the RCS rat and rd mouse*

Gao and Hollyfield (1995) described the upregulation of bFGF mRNA in the ONL of the RCS rat and rd mouse, two strains in which genetic defects drive the photoreceptor dystrophy.

Fig. 10. Edge effects. The green label is an antibody to bFGF protein, the red label is an antibody to GFAP. All retinas are from animals raised conventionally, in dim cyclic light with lights located in the ceiling. **A, B**: bFGF protein levels in the ONL (onl) were consistently very high at the edge of adult retina. In A, the retinal edge has folded during processing, enabling a gradient in bFGF labelling from the extreme edge centrally to be seen in a single image. GFAP levels in Müller cells were also raised towards the edge of the retina. At higher power (B), bFGF is seen to concentrate in ONL somas and to colocalise with GFAP expression in Müller cell processes. **C:** A similar colocalisation of bFGF and GFAP expression was apparent at the edge of the adult C57 mouse retina. **D:** At the optic nerve head, bFGF labels astrocytes in

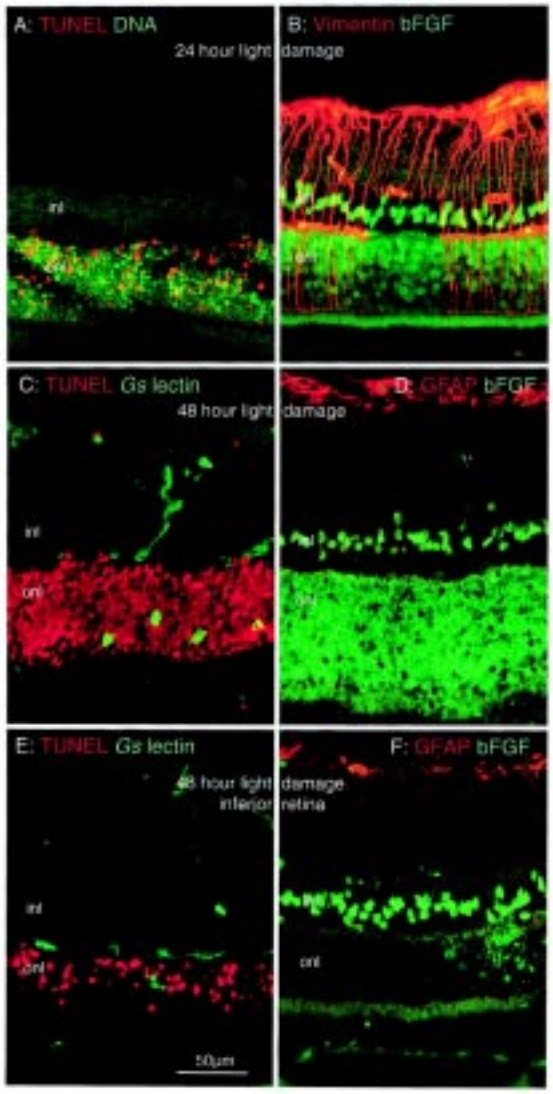

Fig. 11. Damaging light induces an upregulation of bFGF which is related to the damage caused, not to the intensity of the light. **A:** Bright (1400 lux) continuous light (BCL) for 24 h induced fragmentation of DNA in photoreceptors of adult rat retina, seen as red nuclei among those labelled green by a DNA dye. The dying cells are all in the ONL (onl). **B:** The same exposure to BCL induced high levels of bFGF in the retina, most prominently in the ONL. The red vimentin label has been removed over a rectangular patch to show the bFGF labelling more clearly. **C, D:** BCL for 48 h caused TUNEL labelling of apparently all cells in the ONL in superior retina and a massive concentration of bFGF protein in the ONL. The green label in C is the *G. simplicifolia* lectin, which labels vessels and microglia. **E, F:** BCL for 48 h induced death of some ONL cells in inferior retina (E) and a limited and patchy upregulation of bFGF in the ONL (F).

Whether this upregulation occurs because of environmental sequelae of gene malfunction (such as hypoxia in the juvenile RCS rat, Valter *et al.*, 1998) or by some more direct action of the mutation is not established.

3.4. The Price of Protection

The experiments reviewed by Penn and Anderson (1991) created much new understanding, but raised an enigma. It is easy to make teleological sense of their observation that light conditioning upregulates protective mechanisms in the retina. But their data make clear that that upregulation requires levels of light which induce irreversible damage (the loss of photoreceptors) and that the upregulation is reversible. Why might mechanisms of retinal protection evolve with a threshold such that irreversible damage occurs before the protection is effective? Why allow the retina to become vulnerable after it has been made resistant to damage? Why not just turn on the protection available and leave it on? We surmised that the protective mechanisms come at a price, that the price is a loss of sensitivity and that the regulation of the retina's self-protective mechanisms has evolved as a compromise between sensitivity and survival.

3.4.1. *Metabolic suppression*

In approaching the question of how molecules such as bFGF protect neurones from stress we were influenced by the concept of ''metabolic suppression''. Hochachka (1986) reviewed a body of literature which concerned the response of aerobic tissues to hypoxia. All aerobic tissues, it was argued, show a common response to hypoxia: they reduce their oxidative metabolism. The mechanisms of this reduction were considered to include ''metabolic arrest'', the shutdown of oxidative metabolism with a concurrent reduction in function, and ''membrane stabilisation'', effectively a reduction in the leakiness of membranes. Both mechanisms seem available and effective in non-mammals, but are less available in mamma-

lian tissues, which are as a consequence much less tolerant of hypoxia.

The retina's ability to upregulate glycolysis in hypoxia (Section 3.1.3) gives it considerable resistance to hypoxia, in terms at least of the survival of photoreceptors. Nevertheless Steinberg (1987) argued that metabolic suppression does occur in the retina and we have asked whether bFGF, which protects the photoreceptors from damage in many circumstances (Section 3.2) protects by inducing metabolic suppression.

Since much of the high oxygen consumption of photoreceptors goes to fuel the Na^+/K^+ pumps of the inner segments, it seemed possible that bFGF might induce a reduction in the load on that pump by reducing the number of active cGMP-gated channels in the outer segment membrane, thereby reducing its leakiness. This would be a form of the "membrane stabilisation" reaction postulated by Hochachka (1986) as a mechanism of metabolic suppression. Since the Na^+/K^+ pumps function to maintain the dark current of photoreceptors, it seemed possible that bFGF might mediate a reduction in dark current and thereby in the ERG. The idea was tested in two models of bFGF upregulation and the results were not entirely those predicted.

3.4.2. *Endogenous upregulation*

Bush and Williams (1991) described an initially puzzling phenomenon. They showed that section of the optic nerve of the rat eye makes the photoreceptors highly resistant to light damage, the resistance taking 2–3 weeks to develop after the section. As the protective effects of bFGF became established (Section 3.3) Kostyk *et al.* (1994) tested whether bFGF regulation was affected by nerve section; they showed a strong upregulation of bFGF protein in the ONL following optic nerve section. These observations provide a model of endogenous upregulation of bFGF in photoreceptors, associated with a strong protection of the photoreceptors from light damage.

Our observations (Stone *et al.*, 1997) were that nerve section reduces the b-wave of the ERG markedly (Fig. 12A,B) without damaging the general morphology of outer retina (Fig. 12H–J), or reducing its content of key molecules, such as cytochrome oxidase and opsin (Fig. 12J), and confirmed that nerve section upregulates bFGF expression in the ONL (Fig. 12K,L). Nerve section reduced the b-wave recorded in light- as well as dark-adapted conditions (data not shown). The traces in 12A and B were elicited with an 8 msec flash of moderate ($\leq$28 cd/m^2) intensity. When a briefer (1.5 msec) and brighter flash generated by a photoflash unit was used, the early negative a-wave was more apparent (Fig. 12D). Nerve section again caused a major reduction in the b-wave, but the a-wave was not reduced in amplitude.

3.4.3. *Exogenous application*

Nerve section may of course upregulate molecules and mechanisms other than bFGF. To test whether bFGF could account for the ERG changes caused by nerve section, we injected bFGF into the vitreous humour using doses and approaches devised by Faktorovich *et al.* (1990, 1992). Initial experiments were done in the cat eye, whose large size makes delivery easier; in both animals tested a major reduction in photopic ERG was observed (data not shown). The experiment was then extended to the rat, with similar results (Fig. 12C). Again, when a brief intense flash was used to optimise the a-wave it was apparent that injected bFGF suppressed the b-wave but not the a-wave. In our hands the effects of exogenously applied bFGF on the ERG were very similar to those of endogenous upregulation of bFGF.

3.4.4. *By what mechanism?*

The evidence just presented that bFGF suppresses the b-wave but not the a-wave was a surprise, for the idea of metabolic suppression (Section 3.4.1) led us to expect a downregulation of the metabolic activity of the photoreceptors, and therefore presumably of the a-wave. Instead the data indicate that bFGF produces a selective suppression of the b-wave, thus mimicking the

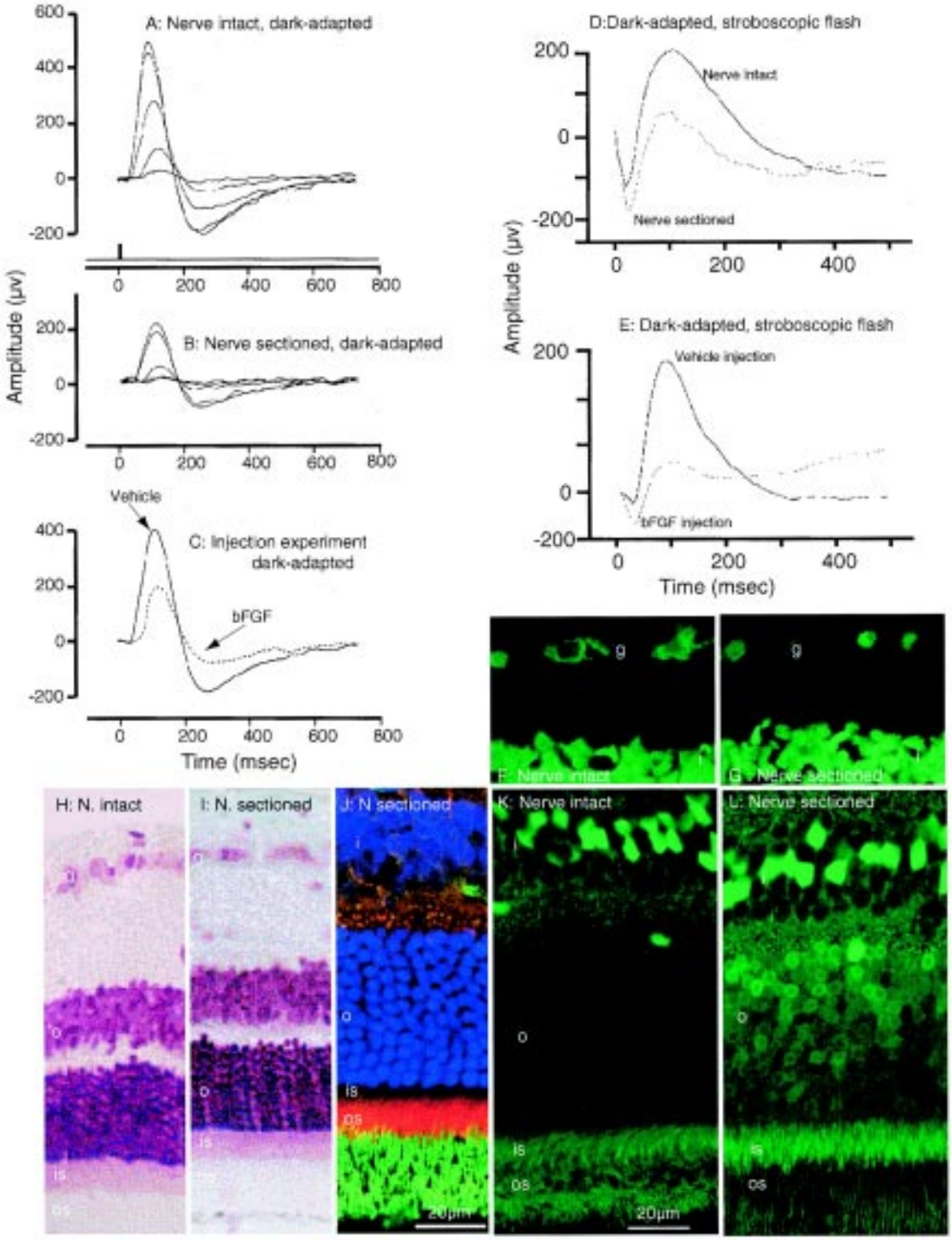

Fig. 12. (*Caption opposite*)

effects of both hypoxia and hypoglycaemia (Winkler, 1983, 1995; Section 3.1.3).

It is possible that the selective loss of the b-wave in the energy-deprived retina represents not a passive response to the deprivation but an active response of the retina, with a protective purpose. bFGF, known to be highly protective to photoreceptors (Section 3.2, Section 3.3) produces the same ERG change, i.e., a selective reduction of the b-wave. Perhaps bFGF, hypoxia and hypoglycaemia all activate the same, still enigmatic, protective mechanism(s).

4. MECHANISMS OF ADULT INSTABILITY: DEARTH OF ENERGY AND EXCESS OF OXYGEN

What mechanisms make the adult retina unstable, despite the natural longevity of mammalian CNS neurones and the self-protective mechanisms the retina has evolved? We review here three distinct forms of retinal destabilisation.

4.1. The Replete Retina Detached: Starvation

4.1.1. *Death, survival and proliferation: reactions to detachment*

Detachment of even a small part of the neural retina from the choroid threatens vision in several ways. The detachment may expand from its initial site, displacing large areas of retina; clinically this risk is limited by prompt surgery (Michels *et al.*, 1990, Chapter 15). Even if the detachment is reduced promptly, however, the re-attached area does not fully recover function. Further, proliferative changes (proliferative vitreoretinopathy) can develop in the re-attached area and spread to involve surrounding, healthy retina, sometimes destroying the whole retina (Glaser and Michels, 1989). What is happening in the detached retina?

Fisher and colleagues have described the reaction of the retina to detachment in cellular and molecular terms, in a feline model (Anderson *et al.*, 1983; Erickson *et al.*, 1983; Cook *et al.*, 1995; Fisher *et al.*, 1991; Lewis *et al.*, 1996; Lewis *et al.*, 1998).These studies showed that when the retina is detached from the choroid:

- There ensues a wave of death among retinal neurones which is largely specific to photoreceptors (Erickson *et al.*, 1983).
- The surviving photoreceptors dismantle their outer segments, and the organelle concentrations of the inner segment become sparse (Anderson *et al.*, 1983).
- The surviving photoreceptors appear to withdraw the terminals of their axons from the OPL (Erickson *et al.*, 1983; Lewis *et al.*, 1998).
- Glial cells of the retina proliferate (Fisher *et al.*, 1991) and **hypertrophy**, and their processes spread along both outer and inner surfaces of the retina (Fisher *et al.*, 1991).

The changes which occur in human retina when detached have not been established in such detail; Wilson and Green (1987) and Chang *et al.* (1996)

Fig. 12. bFGF caused a reduction in the b-wave of the ERG, whether applied exogenously or upregulated endogenously. **A, B:** Dark-adapted ERGs recorded from the vitreous humour in a rat in which one optic nerve had been sectioned three weeks previously, to an 8 msec flash of 5 intensities (0.11, 0.56, 5.04, 14 and 28 cd/m^2). The response was consistently smaller from the nerve sectioned eye (A vs B). These responses, and those in C, are dominated by the positive (upward-going) b-wave of the ERG. **C:** The injection of bFGF (1 μg) into the vitreous humour produced a similar reduction of the ERG, apparent 1–10 days later. C compares responses from bFGF-injected and vehicle- (PBS-) injected eyes of the same animal to an 8msec flash of intensity 28 cd/m^2. **D:** When a brief (1.5 msec), bright photoflash was used, the early negative a-wave was more apparent. Nerve section again caused a major reduction in the b-wave but an apparent increase in the amplitude of the a-wave. The increase may only be apparent, the result of the reduction of the b-wave. **E:** A similar reduction of the b-wave, and apparent increase in the a-wave, were seen 1–2 days after injection of bFGF into the vitreous humour. In F–L, *g* denotes the ganglion cell layer, *i* the inner nuclear layer, *o* the outer nuclear layer, *is* the inner segments, *os* outer segments. **F, G:** Nerve section reduced the size of somas in the ganglion cell layer (g), presumably by causing retrograde degeneration of ganglion cells. **H, I:** Except for this effect on ganglion cells, the histology of the retina, including the inner and outer segments (is, os) appeared normal in the nerve-sectioned eye. **J:** An antibody to cytochrome oxidase (red) confirms that nerve section did not reduce the concentration of this enzyme in inner segments. An antibody to rod opsin (green) indicates that the outer segments appeared of normal length. **K, L:** Nerve section increased bFGF concentration in the retina (confirming Kostyk *et al.*, 1994), most prominently in the cells of the ONL.

have shown however that, as in the cat, detachment of the human retina causes the death of photoreceptors. Berglin *et al.* (1997) have shown that in the rabbit, as well as the cat and human, detachment causes the death of photoreceptors.

Death of and damage to photoreceptors will degrade the visual function of the retina in obvious ways. The glial proliferation may be the basis of proliferative vitreoretinopathy and may therefore be the most threatening aspect of the retina's response to detachment.

4.1.2. *Mitigating the retina's reaction to detachment*

Detachment separates the outer retina (the photoreceptors) from the source of its prime nutrient (glucose) and from its source of oxygen which, via the TCA cycle, greatly increases the efficiency of glucose metabolism (Section 3.1). Detached retina is presumably both hypoxic and hypoglycaemic. We have tested whether the effects of detachment can be mitigated by increasing the flow of oxygen from the choroid to the retina. Such an increase can be achieved by placing the animal in oxygen-enriched air (Alder and Cringle, 1985; Linsenmeier and Yancey, 1989). To test this idea we created (following Anderson *et al.*, 1986) retinal detachments in cat eyes and observed the state of the retina after three days. Some were kept for those three days in normoxia, others in 70% oxygen (hyperoxia). Hyperoxia

- reduced the death of photoreceptors (Fig. 13A–C). This reduction was evident in five of seven experiments, and was the least reliable of the effects of hyperoxia.
- slowed the disintegration of synaptic structures in the OPL (D–F);
- stabilised bFGF distribution in the detached retina (G–I);
- stabilised the morphology of the outer segments of photoreceptors, (J–L);
- reduced the loss of cytochrome oxidase from the inner segments, (J–I);
- reduced the accumulation of glutamate in retinal neurones and glia (M–O); and
- suppressed the proliferative glial response to detachment.

  Specifically, detachment without oxygen supplementation caused a glial proliferation, seen as nuclei labelled with an antibody to the nuclear antigen Ki-67 (following Geller *et al.*, 1995). Oxygen supplementation reduced the density of mitotic nuclei from 32.5 per mm of section (20 μm thick) to 5.5 with supplementation. (Mitotic nuclei were rare in normal, attached retina).

These results suggest that the detached retina is both hypoxic and hypoglycaemic, because it is detached from its source of both and cannot switch (Section 3.1) to oxidative metabolism in response to the hypoglycaemia, nor to glycolysis in response to the hypoxia. Oxygen supplementation is protective presumably because it allows an upregulation of the oxidative metabolism of the residual glucose available in the detached retina. Glucose supplementation should prove to be similarly protective, but the point has yet to be tested. Present results also suggest (Section 6.1) that offering patients oxygen (and glucose) between diagnosis of a detachment and surgery should improve the function of re-attached retina and reduce the possibility of proliferative vitreoretinopathy.

4.2. The Depleted Retina Attached: the "Oxygen Toxicity" Hypothesis

In their analysis of the *rds* dystrophy, Travis *et al.* (1991) suggested that in the absence of outer segments (which fail to form in the *rds* homozygote) "*the photoreceptors may…be degenerating from prolonged O_2 toxicity*". Two of our own observations have led us to adopt and broaden this suggestion to predict that any substantial depletion of photoreceptors threatens the survivors by exposure to toxic levels of oxygen. First, in an analysis of the RCS dystrophy, we noted that hypoxia during the critical period accelerates the dystrophy but hypoxia after the critical period is protective (Valter *et al.*, 1998). Second, we observed that hypoxia is protective against light damage to adult retina (Section 4.3).

Seeking to understand how hypoxia could be a toxin for the developing retina and a tonic for the

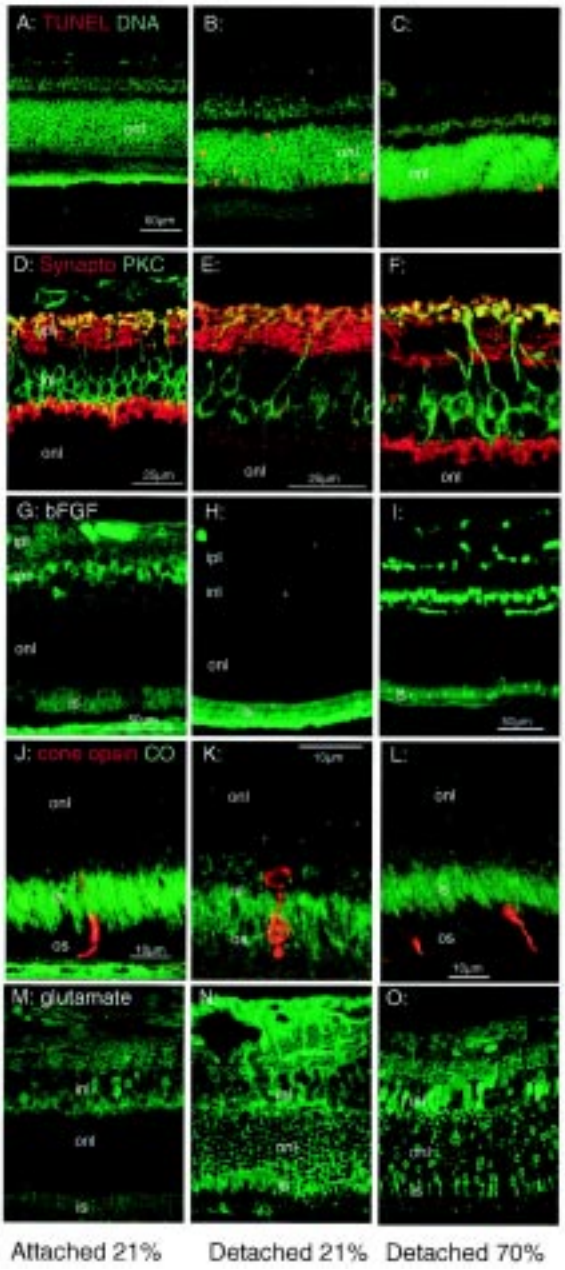

Fig. 13. Oxygen supplementation mitigates the damage caused by retinal detachment. **A–C:** Detachment induced death (red labelling) among cells of the ONL (B). The rate of death was reduced by oxygen supplementation (C). This result was obtained in five of seven experiments. Death is zero in attached retina (A). The scale in A refers also to B and C. **D–F:** Detachment reduced the labelling of synaptophysin in the OPL (compare D and E). The level of synaptophysin in the ONL was stabilised by oxygen supplementation (F). The scale in E applies also to F. **G–I:** Detachment caused a downregulation of bFGF protein, normally found in all layers of the retina (compare D and E). Some bFGF appears to have concentrated in the inner segments (is) of the detached retina. Oxygen supplementation stabilised bFGF distribution within the detached retina (F). However, the blobs of bFGF in the inner segments (is in I) are abnormal. The scale in G also refers to H. **J–L:** In attached retina (J) cone opsin (red) concentrates in the outer segment of a cone, with a blush of staining in its soma. The green fluophore marks cytochrome oxidase in the inner segments. Detachment caused a dismantling of the outer segments of cones and the appearance of cone opsin in cone somas (compare J and K). Oxygen supplementation stabilised cone opsin in outer segments and reduced the dispersion of cytochrome oxidase from the inner segments (L). **M–O:** Glutamate was detected by a rabbit polyclonal antibody donated by R. E. Marc, following Kalloniatis *et al.* (1996). Detachment caused a rise in glutamate levels in all layers of retina, most prominently in the processes of Müller cells (M, N). Oxygen supplementation reduced this rise (O).

depleted adult retina, we reasoned as follows. Any toxic effect of hypoxia on photoreceptors should be self-*limiting* because the death of some would reduce oxygen consumption in the outer retina and relieve the hypoxia. Prolonged hypoxia should produce a retina in which the photoreceptor population is depleted but stable. In a retina depleted of photoreceptors, by contrast, the outer retina is (we argued) hyperoxic. The oxygen comes from its normal source, the choroid, and is in excess because many photoreceptors, having died, have stopped using oxygen and the choroid does not autoregulate (Section 1.1.4). Further, the toxic effect of hyperoxia on those survivors should be self-*reinforcing*. The loss of some photoreceptors would reduce oxygen consumption in the outer retina, add to the hyperoxic stress on the survivors and drive the dystrophy to completion. The idea was tested in three models.

4.2.1. *The RCS rat*

RCS rats were raised in normoxia to the age of P27, thus through the critical period in which there is a major depletion of rods. Some were then raised in normoxia, others in 10% oxygen, for up to 43 days. Hypoxia slowed the rate of

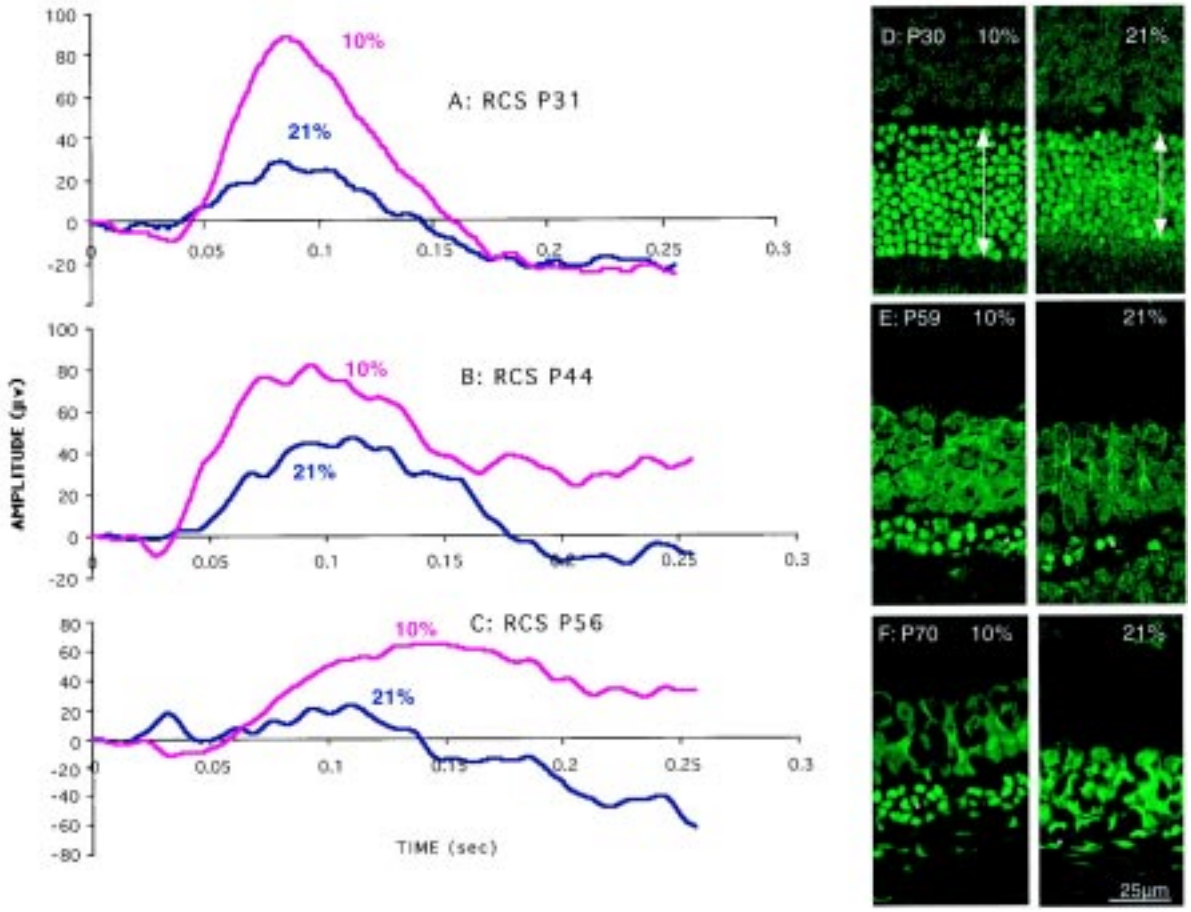

Fig. 14. Hypoxia slowed the late stages of the RCS dystrophy. **A–C:** The dark-adapted ERG (recorded from the cornea, in response to a stroboscopic flash) was larger in pups raised in hypoxia from P25. The ERG declined with age (B and C) but was consistently larger in the hypoxia-raised pups. **D–F:** Hypoxia from P25 also slowed the reduction of the thickness of the ONL in this strain. The arrows and the asterisks mark the ONL.

ERG loss in this strain (Fig. 14A–C). Three morphological features of the RCS dystrophy were reduced by hypoxia: the loss of cells from the ONL (Fig. 14D–F) was slowed; the loss of opsin bearing outer segments (both rod and cone) was slowed; and the upregulation of bFGF which occurs during the dystrophy was reduced. All three morphological trends could contribute independently to the preservation of the ERG.

The effect of hypoxia in reducing the upregulation of bFGF in the ONL which normally occurs during the RCS dystrophy suggests that the hypoxia relieves stress on the photoreceptors (Section 3.3), i.e., that hypoxia acts "upstream" from the regulation of bFGF, as well as of photoreceptor damage and death.

4.2.2. *The rd mouse*

rd mice were raised in normoxia until P15, at which age the rod population of the photoreceptors is severely depleted, and then in either normoxia or 10% oxygen for up to 18 more days. Hypoxia slowed the loss of cone opsin bearing outer segments, slowed the loss of the last row of cells from the ONL and slowed the deterioration of the ERG.

4.2.3. *After light-induced depletion*

Following Noell *et al.* (1966) we used bright continuous light (BCL) to deplete the photo-

receptor population in adult, genetically normal retinas. Exposure of young adult albino rats to bright (1400 lux) illumination from above for 48 h induced death of all photoreceptors in a "sensitive patch" of superior retina (also apparent in the results of Duncan and O'Steen 1985 and Wiechmann and O'Steen, 1992) and caused the death of lesser numbers of photoreceptors throughout the rest of the retina (Fig. 8).

Most photoreceptors were TUNEL$^-$ at the end of BCL, suggesting that BCL killed only a minority of cells. Nevertheless, confirming many previous reports, the retina examined 14 days later was devoid of photoreceptors except for a few at the edges of the retina (Fig. 15E). Among these survivors, moreover, marked degenerative changes were evident, including shortening and distortion of the outer segment and opsin accumulation in their somas. When the pattern of degeneration between 0 and 14 days after BCL was mapped (Fig. 15C–E) it was apparent that the depletion spread peripherally from the "sensitive area". At 3 days, the sensitive area was denuded of photoreceptors, surrounding areas of the ONL were thinned and opsin labelled outer segments had also disappeared from a wide surrounding area. After 7 days these degenerative changes had spread more peripherally until, at 14 days, photoreceptors persisted only at the retina margin.

The spread of degeneration identified in Fig. 15 suggested that a toxin spreads from the sensitive region into surrounding retina. We hypothesised that the toxin was oxygen, unused by the photoreceptors destroyed by BCL. To test this we kept some animals post-BCL in 10% oxygen, some in 70% oxygen. In those kept in hyperoxia, the degeneration was greatly accelerated, reaching the edge of the retina by 3 days instead of 14 days (Fig. 16A–D). Conversely, in those kept in hypoxia, post-BCL degeneration was markedly slowed (Fig. 16E–I). ERG recordings 5 days post-BCL showed apparently complete loss of the response in animals kept in normoxia, but a clear response from animals kept in hypoxia (Fig. 16G).

Two novel conclusions about light damage, at least in the present model, would seem to follow. First, most photoreceptor death precipitated by BCL is caused, not by the BCL, but by a toxin which attacks the surviving photoreceptors from the region of greatest BCL-induced damage. Second, that toxin is oxygen.

4.2.4. *A general mechanism of late-stage dystrophies?*

The observations just presented support the hypothesis that photoreceptors killed by a mutation or environmental stress leave behind a factor toxic to surviving photoreceptors, and that the factor is oxygen. It seems possible, arguably likely, that this mechanism operates in the late stages of all photoreceptor dystrophies, including human RP. The present suggestion was anticipated by two previous studies. Travis *et al*. (1991) suggested that oxygen toxicity drives the *rds* dystrophy (Section 4.2). Fain and Lisman (1993), Lisman and Fain (1995) proposed a general "equivalent light" hypothesis of photoreceptor dystrophies, central to which is the suggestion that in many dystrophies photoreceptors die because they are chronically activated. They discussed several mechanisms which might lead from chronic activation to death, among them that "degeneration could result from a change in the oxygen tension in the outer retina". In broad stroke and close detail however their equivalent light hypothesis is distinct from the present suggestion that dystrophies occur by depletion of some photoreceptors followed by a depletion-induced oxygen toxicity to the survivors. The present suggestion is set out formally in Section 5.

4.3. The Continuing Enigma of Damage by Continuous Light

Since the vulnerability of genetically normal retina to bright continuous light (BCL) was described by Noell (Noell *et al*., 1966), the phenomenon has attracted great interest and much experiment, yet its mechanism remains elusive (for recent reviews see Organisciak and Winkler (1994) and Reme *et al*. (1996)). We have tested the effect of oxygen levels during BCL on

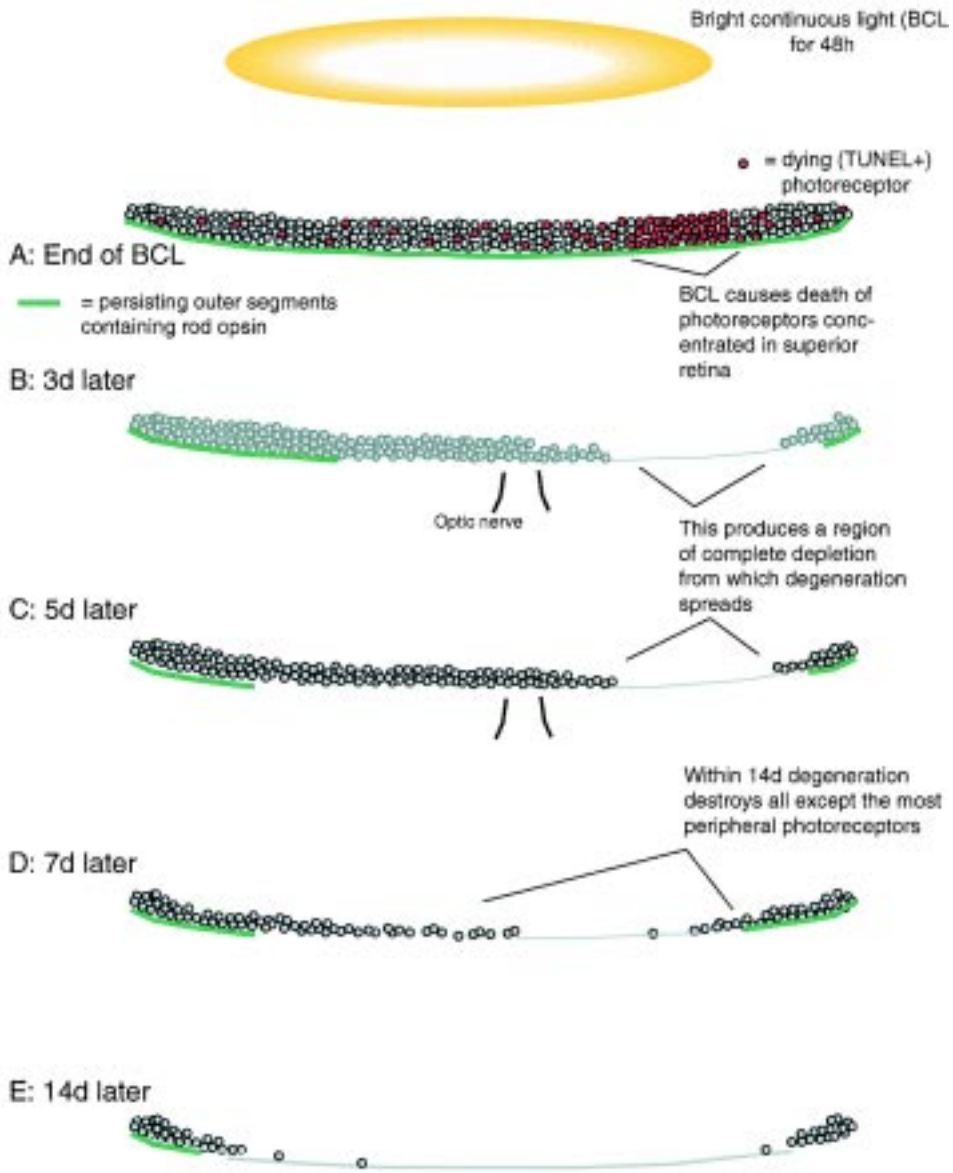

Fig. 15. Much of the dystrophy induced by BCL occurs after the end of exposure to BCL. The drawings represent the ONL of the retina and (green line) the presence of opsin-labelled outer segments. **A:** In the retina of an albino rat raised in normal cyclic light (40 lux, 12 h on/12 h off) an episode of BCL (1400 lux for 48 h) induced the death of photoreceptors, most markedly in a ''sensitive patch'' in superior retina. **B:** 3 days later, the photoreceptors in the sensitive patched had fully degenerated and a reduction in the thickness of the ONL was apparent more peripherally. Opsin bearing outer segments had also disappeared from a wide region around the patch denuded of photoreceptors. **C–E:** Over the next 11 days, degeneration—loss of ONL cells, loss of outer segments in the survivors—spread from the sensitive patch towards the edges of the retina.

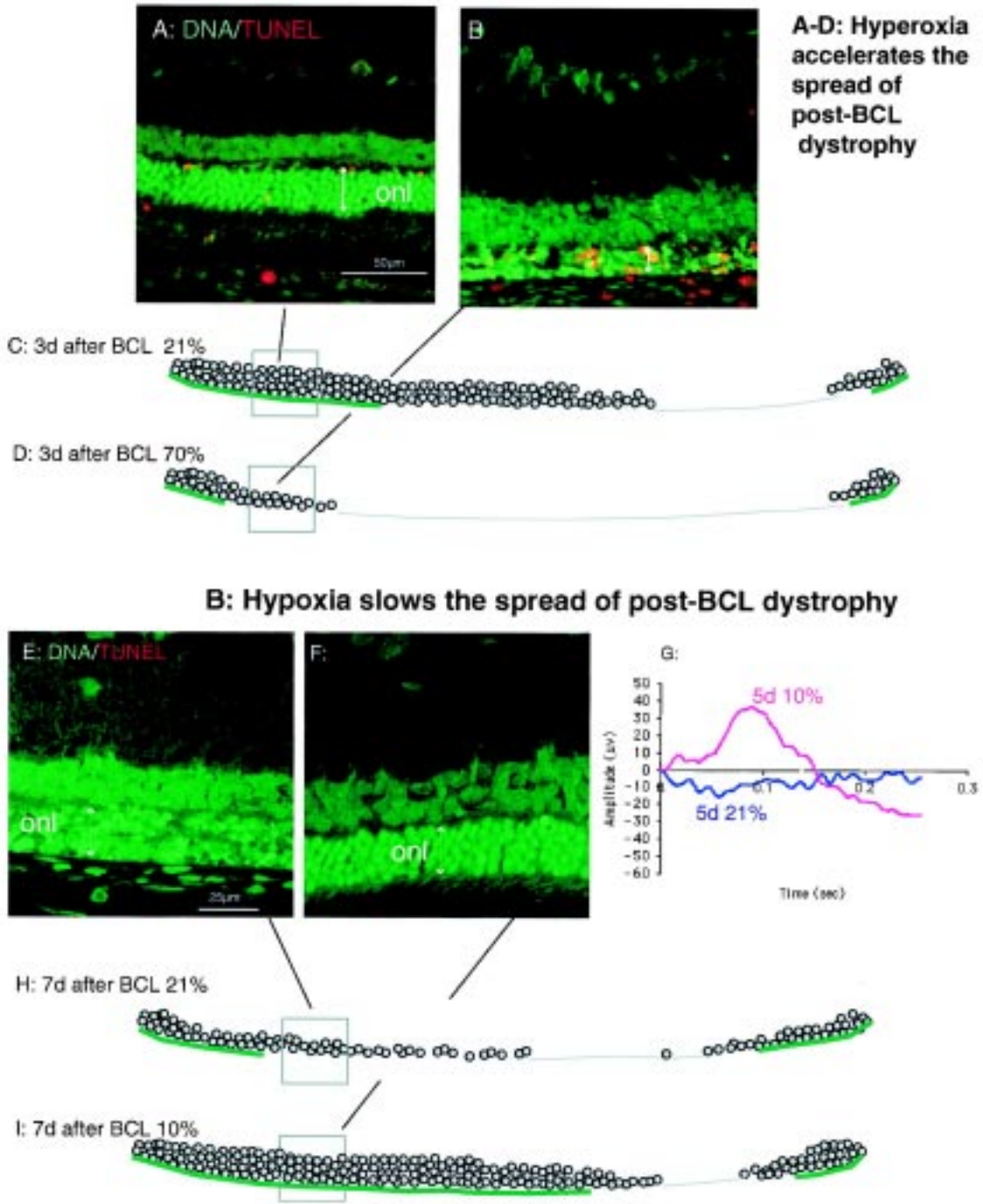

Fig. 16. Hyperoxia accelerates and hypoxia slows post-BCL degeneration. **A–D:** In animals kept in 70% oxygen after BCL, the spread of degeneration from the sensitive patch towards the edges of the retina was accelerated, as shown schematically in C and D. A and B compare the histology of the retina in the inferior mid-periphery. This region was largely intact in the animal kept in normoxia; in the animal kept in hyperoxia the ONL had degenerated. **E–I:** In animals kept in hypoxia after BCL exposure, the spread of degeneration from the sensitive patch was slowed, as shown schematically in H and I. E and F compare the histology of the retina in the inferior mid-periphery. The ONL in this region was largely degenerate in the animal kept in normoxia (E). In the animal kept in hypoxia after P25 the ONL in the same region was largely intact (F). At 7 days post-BCL, no ERG was detectable in the normoxic animal (blue trace in G) but an ERG persisted (red trace, b-wave maximum of 25 μv) in the hypoxic animal (red trace).

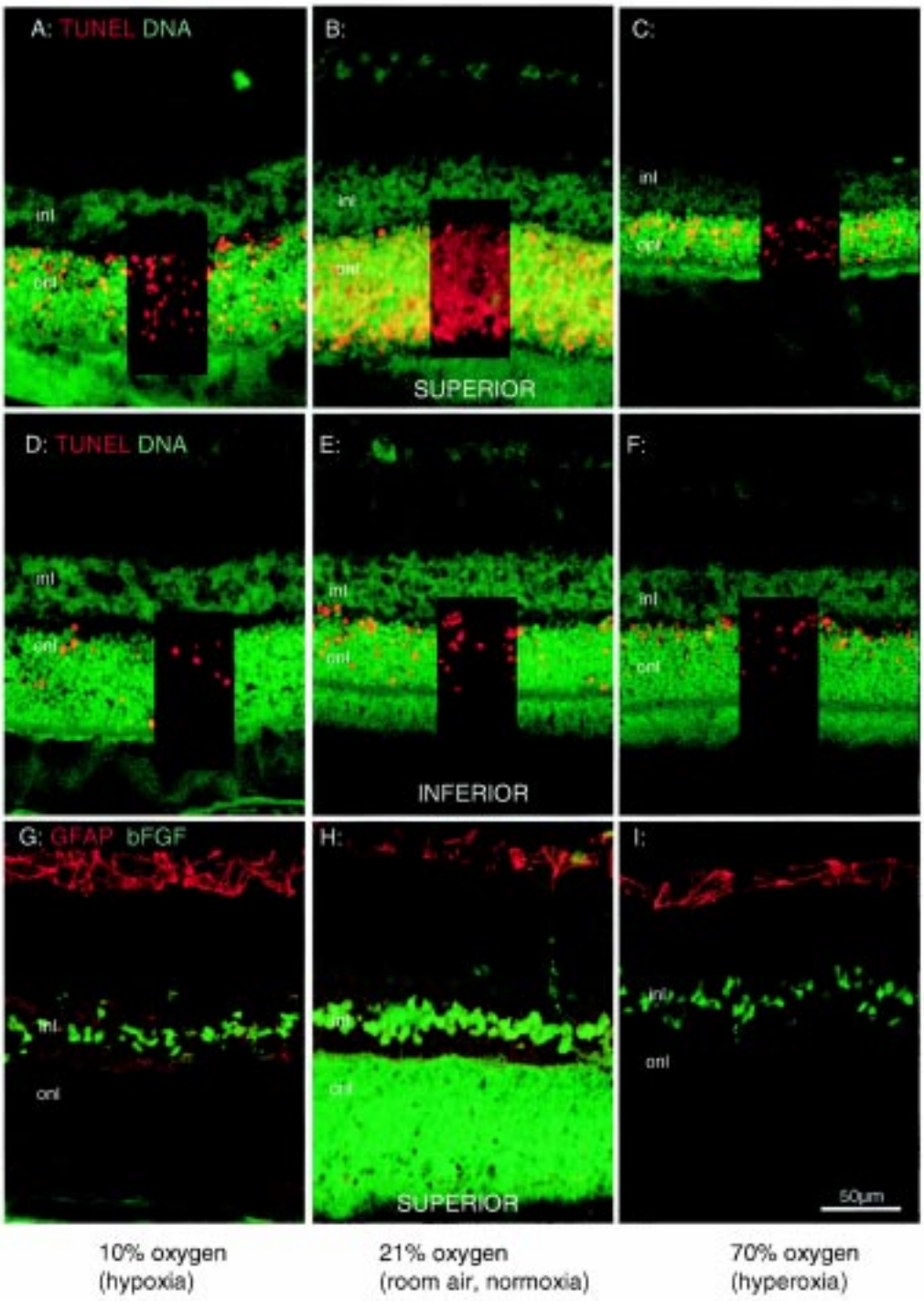

Fig. 17. Dual rescue effect of oxygen during BCL. **A–C:** In rats raised in dim cyclic skylight and then exposed to BCL for 48 h in normoxia, degeneration was intense in the superior mid-periphery (B, red label of $TUNEL^+$ cells). Both hypoxia (A) and hyperoxia (C) reduced the degeneration induced by BCL. **D–F:** In the inferior mid-periphery, the degeneration induced by BCL was less than in the superior mid-periphery (E), as previously noted (Fig. 8, Fig. 10 and Fig. 15). Again the degeneration caused by BCL was reduced by both hypoxia (D) and hyperoxia (F). **G–I:** BCL induced a strong upregulation of bFGF protein in the ONL of superior mid-peripheral retina (H), as previously noted (Fig. 10). That upregulation was reduced by both hypoxia (G) and hyperoxia (I). Thus the rescue effects of oxygen were not mediated by bFGF.

the photoreceptor death induced in the albino rat retina by bright continuous light.

The effects were surprising. Both hypoxia and hyperoxia reduced the level of cell death induced by BCL (Fig. 17A–C, D–F) and both slowed the upregulation of bFGF also induced by light damage (Fig. 17G–I).This dual protective effect suggests that light damage results from two processes, one involving hypoxic toxicity and the other hyperoxic toxicity. It seems unlikely that both mechanisms could operate simultaneously; one possibility is that one process acts during part of the day-night cycle, the other during the other half of the cycle.

The importance of circadian rhythms in the phenomenon of light damage has been demonstrated by Duncan and O'Steen (1985) and Organisciak *et al.* (1998b), who presented evidence that the retina is most sensitive to light damage in dark half of the day/night cycle. The retina has strong circadian and light-driven rhythms in its secretion of local neurohormones, in particular dopamine (released by amacrine cells during "day") and melatonin (released by photoreceptors during "night") (reviewed in Zawilska, 1994; Morgan and Boelen, 1996a,b). Wiechmann and O'Steen (1992) reported that exogenously applied melatonin exacerbates light damage and Bush *et al.* (1997) reported that selective blocking of melatonin receptors protects against light damage. Given the vulnerability of the retina in the presence of melatonin, it can be readily understood that the light-induced release of dopamine protects the retina from light damage, because dopamine suppresses melatonin secretion. What is presently mysterious is the role of melatonin. The vertebrate retina evolved in a reliable day-night cycle of light; somewhere in the mechanisms it evolved to adapt to that cycle lies the key to the damage caused by BCL.

Finally we stress that this dual protective effect of oxygen is found only when hypoxia or hyperoxia are applied *during* BCL. The degeneration which follows BCL appears to be driven by oxygen toxicity only; in that period hypoxia is protective, and hyperoxia is highly toxic (Section 4.2.3).

5. A TWO STAGE MODEL OF RETINAL DYSTROPHIES

The ideas and data presented in Section 4 lead to the following model of photoreceptor dystrophies (other than the dystrophy which follows detachment).

5.1. Proposal

It is proposed that dystrophies occur in two stages, labelled (1) and (2) in Fig. 18. First a lethal mutation and/or environmental factor, such as excess light or stress during the critical period, causes depletion of the photoreceptor population. This depletion reduces oxygen consumption in the ONL, causing oxygen levels in the layer to rise. The hyperoxia is toxic, exacerbating the depletion ((2) in Fig. 18). The continuing death of photoreceptors exacerbates the hyperoxia, driving the dystrophy to exhaustion. The mechanisms which cause photoreceptor death at the two stages are thus very different. The first stage may be specific to the individual, a particular mutation or a particular environmental episode. The second stage is driven by a common mechanism, oxygen toxicity.

The model includes one further element. Photoreceptors which survive the oxygen toxicity are damaged by it and in response upregulate (Section 3.3) their expression of protective factors, such as bFGF (Section 3.2–4), or CNTF (LaVail *et al.*, 1992b, 1998; Wen *et al.*, 1995), which slow the degeneration. The rate of photoreceptor loss is the net result of damage and self-protection.

The model has evident limitations. In particular, its central tenet that outer retina becomes hyperoxic after photoreceptor depletion has yet to be tested by direct measurement. It is useful because it provides testable explanations. It can explain, for example, why cones die in dystrophies caused by mutations in rod-specific genes; why many dystrophies progress relentlessly; why the progress of dystrophies can be slow (the protective mechanisms). Further, the vulnerability of photoreceptors during their critical period (Section 2.4) explains why many rodent dystro-

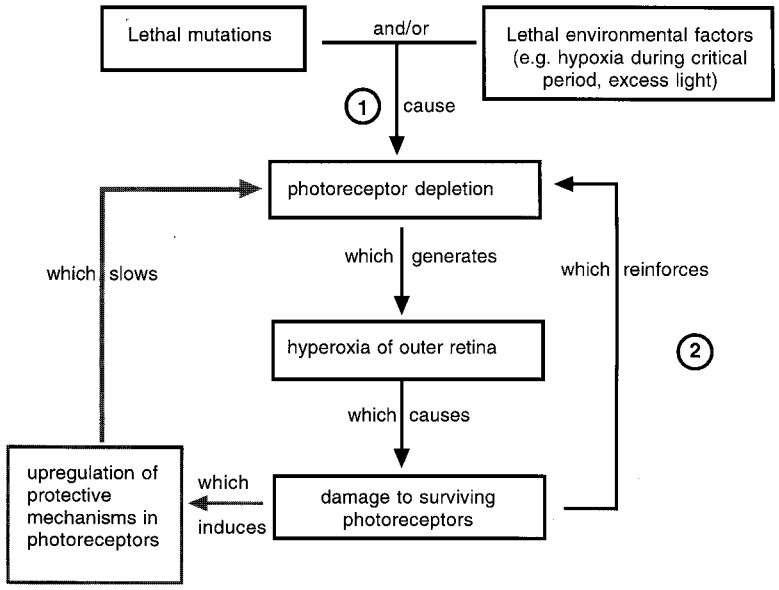

Fig. 18. A model of the pathogenesis of photoreceptor dystrophies. It is proposed that the dystrophies occur in two stages, "initial depletion", labelled **1**, and "late", labelled **2**.

phies begin in the critical period (Section 2.4.2.1). If human dystrophies also begin in the critical period, understanding is gained of why the onset of human dystrophies is so insidious, for the human critical period occurs at an age when the infants' visual capacity and motor skills are undeveloped (Section 2.5), and the gathering blindness goes (we suggest) unnoticed amid the general immaturity. The model also makes testable predictions about therapies which, drawing on the early and late stages of the degeneration, are to some extent novel (Section 6.3.1).

In epistemological terms, we stress again (as in Chapters 4 and 5 of Stone, 1983) that such models are only as valuable as their ability to yield predictions capable of disproof. Section 6 sets out a number of predictions, formulated as approaches to therapy.

5.2. Comparative Note: How Epithelia Self-Protect

The mechanisms which (Fig. 18 proposes) cause photoreceptor dystrophy—those of a critical period of vulnerability of a single class of cell, the dozens of mutations lethal to the same class of cell, the impact of a massive but poorly regulated circulation, the self-reinforcing feature of oxygen toxicity—seem unique to the retina. One feature of the present model has interesting analogues in other tissues.

The skin, like the retina, is exposed to radiant energy and, as in the retina (Section 3.2), that energy upregulates mechanisms (light absorbing pigments) which protect against later exposure. The epithelium of the bronchi and lung is exposed to inhaled gases and is the major site of damage by the prolonged inhalation of high concentrations of oxygen (bronchopulmonary dysplasia, O'Brodovich and Mellins, 1985). In response to an episode of hyperoxia, the bronchopulmonary epithelium upregulates its antioxidant mechanisms and becomes more resistant to subsequent episodes of hyperoxia (Clerch and Massaro, 1993; Clyde *et al.*, 1993). As with the skin, the protection provided is not immediate, but requires hours to days to develop.

All three epithelia are exposed to damaging radiation or toxins and all have developed mechanisms of self-protection which are prospective. Each constantly uses its recent experience to prepare itself for future exposure and, with that conditioning, can last the life of the organism. For each, an episode of unprecedentedly high levels of ultraviolet light (for the skin), of visible light (retina) or oxygen (lungs) can be highly damaging.

6. FUTURE DIRECTIONS: PREDICTIONS OF THERAPY

6.1. Retinal Detachment: Oxygen Supplementation Will Improve Outcomes

The retina's response to detachment is the degeneration of some photoreceptors, degenerative changes in the survivors and a glial proliferation (Section 4.1). From the evidence set out in Section 4.1.2 we predict that oxygen supplementation between diagnosis and surgery will be of benefit in corresponding ways: it will rescue photoreceptors in the detached portion of retina, preserve the structure of surviving photoreceptors and reduce the proliferation of glial cells. The clinical outcome should comprise improved visual responsiveness of the re-attached retina and a reduced incidence of proliferative vitreoretinopathy.

6.2. Preventing Photoreceptor Depletion

The photoreceptor dystrophies known as retinitis pigmentosa are not related to detachment. They occur with the retina in place, close to its source of nutrients (the choroid) and, we suggest, involve the two stages set out in Fig. 18.

6.2.1. *Improved perinatal care will prevent some RP*

The age of onset of human dystrophies is not known. Many are not diagnosed until the teen years or young adulthood or (less commonly) until middle age, suggesting that the disease is "adult-onset". When children are screened by the study of their ERG, however, RP can be diagnosed in early childhood (Berson, 1971), suggesting that some forms of the disease begin much earlier. In many animal models of genetically driven photoreceptor dystrophies, the death of photoreceptors begins with the critical period (Section 2.4.2.1). It is possible, arguably likely, that in many forms of human RP the death of photoreceptors also begins in the critical period, and therefore around birth (Section 2.5), making the perinatal period an important stage for intervention.

Approximately 50% of human cases of RP occur without a family history and are termed "simplex" RP (Heckenlively, 1988) and it seems possible that perinatal stress (hypoxia, jaundice, hypoglycaemia, prolonged delivery) might cause a depletion of photoreceptors which leads (via the mechanisms summarised in Fig. 18) to a clinical presentation like that which follows a genetically induced depletion.

Some "simplex" cases of RP are in fact genetic, caused by fresh or re-emerging recessive mutations (Heckenlively, 1988), and there is currently debate over how many are really genetic. Most debates over whether the genesis of a disease lies in nature or nurture lead to acceptance that both genetic and environmental factors are involved, the challenge being to identify their different contributions to the disease. A recent example of such a debate is that over myopia (Wallman, 1994; Mutti *et al.*, 1996).

More generally, the idea that pre- or perinatal stress can cause diseases normally considered "adult-onset" has been argued and developed in studies of foetal and perinatal correlates of human diseases such as hypertension, coronary disease and diabetes (Barker *et al.*, 1995). It would seem valuable now to establish the age of onset of human photoreceptor dystrophies, and to examine whether there is any correlation between perinatal stress and some forms of photoreceptor dystrophy. If a correlate emerges, the possibility of prevention by improved perinatal care will deserve testing.

6.2.2. *Other approaches to preventing depletion must be applied early*

6.2.2.1. *Gene therapy*

Gene therapy has emerged as a possibility for RP as for many other diseases because in this decade molecular geneticists have achieved the identification of the causative mutations at the codon level. Successful gene therapy for retinal dystrophies has been reported in two animal models, the *rd* mouse (Bennett *et al.*, 1996) and the transgenic P23H rat (Peterson *et al.*, 1998; Lewin *et al.*, 1998). The results are technically stunning, with obvious promise for particular forms of RP. In all these studies the investigators knew the age of onset of the rodent dystrophy and delivered the therapy early, during the critical period. If human RP begins with the critical period, around birth (Section 2.5), it may prove important to deliver gene therapy in late foetal life.

6.2.2.2. *Trophic factor therapy*

Trophic factors such as bFGF and CNTF protect photoreceptors from the degeneration which occurs during the critical period in genetically dystrophic species such as the RCS rat (Faktorovich *et al.*, 1990) and *rd* mouse (LaVail *et al.*, 1998). Again, these investigators knew (because they had demonstrated) the age of onset of the dystrophies and delivered the trophic factors in the critical period. Again it may prove important, to prevent depletion of human retina and the sequela of oxygen toxicity, to deliver these factors in late foetal life.

6.3. Therapy for the Depleted Retina

"...there may be common features of the disease at its later stages.. (which) offers the hope that a single form of therapeutic intervention might be able to retard the progress of the disease...delay the loss of cones...and prevent blindness". Lisman and Fain (1995).

6.3.1. *Predictions from the hypothesis of oxygen toxicity*

The idea that the late stages of many photoreceptor dystrophies are driven by oxygen toxicity of outer retina suggests three approaches to therapy.

6.3.1.1. *Hypoxia will slow late stage dystrophy*

No group has tried hypoxia as therapy for RP or, as far as we are aware, as therapy for anything. Empirically, hypoxia slows late stages of the RCS, *rd* and light induced dystrophies (Section 4.2) and we predict that it will slow the late stages of human dystrophies.

Side effects: Knowledge of the side-effects of hypoxia comes from the study of human responses to high altitudes. The percentage of oxygen in air remains constant (at 21%) with altitude but atmospheric pressure and the partial pressure of oxygen decrease. Above about 3000 metres (10 000 ft, pO_2 equivalent to 14% oxygen at sea level) morbidity becomes common (Butler *et al.*, 1992). The symptoms, collectively termed "altitude sickness", include headache, nausea, fatigue, insomnia, reversible total blindness and retinal bleeding. The threshold altitude for and severity of altitude sickness vary markedly however, particularly with rate of ascent.

By contrast, mild hypoxia appears to be largely free of side effects. Every day several million humans experience mild hypoxia as passengers in commercial airliners. These craft are typically pressurised to 8000 ft, and for the duration of the flight (less ascent and descent) passengers experience a partial pressure of oxygen equivalent to 15–16% oxygen at sea level. Air-crew experience the same conditions on a daily basis and are subject to frequent medical examination. There are few reports, if any, of flight-related morbidity among either passengers or crew. And high altitude cities like Mexico City (7350 ft) grow populous and host Olympic games.

Mechanism: Among the adaptive mechanisms which enable animals and humans to tolerate mild hypoxia is the autoregulatory response of capillary beds. If arterial pO_2 drops and the tissue supplied becomes hypoxic, the vessels deliver greater volumes of blood; and vice versa. The choroidal circulation lacks this ability to autore-

gulate (Section 1.1.4) and, we now argue, this lack of regulation is a major element in the cause of the late stages of photoreceptor dystrophies. But conversely, the inability of the choroid to autoregulate means that when the animal breathes oxygen-enriched or depleted air, the excess or lack of oxygen affects outer retina more strongly than other, autoregulating tissues. Thus the factor which causes the hyperoxia localised to outer retina which (we argue) drives the late stages of dystrophies can be used to reduce that hyperoxia.

Delivery: The practicalities of delivering hypoxia remain a challenge. Many of the technologies developed to deliver and control hyperoxia (gas mixtures, selective membrane pumps, mask systems) would seem to be readily applicable, *mutatis mutandis*.

Unknowns: Two deserve mention. It is emerging from the literature on light damage (Section 4.3) that the vulnerability of photoreceptors to damage varies markedly with diurnal phase. It may prove as effective, even more effective, to deliver a hypoxia at certain times of day or night. Second, if Fig. 18 is correct in suggesting that late stage dystrophies are slowed by protective mechanisms induced by the toxin, then reducing the toxin will downregulate the protection, making the retina more vulnerable. It may prove to be important that treatment once commenced be maintained.

6.3.1.2. *Antioxidants will slow late stage dystrophy*

If the late stages of photoreceptor dystrophies are driven partly or wholly by oxygen toxicity, then antioxidant molecules should slow their death. Several authors (Phelps, 1986; van der Hagen *et al*., 1993; Kretzer *et al*., 1984) have reported evidence of a role of antioxidants in other retinal disease. In relation to photoreceptor dystrophies, Penn *et al*. (1987) reported evidence (upregulation of scavenger molecules in light-damage resistant retina, Section 3.2.1.2) that antioxidants play a role in the protection of photoreceptors against light-induced dystrophy and Organisciak *et al*. (1998a) have reported that a systemically delivered anti-oxidant is protective against light damage. Mittag *et al*. (1997) reported that mice in which the activity of a major antioxidant enzyme (superoxide dismutase) was reduced by a transgene are abnormally sensitive to damaging light, implying a normal role for this enzyme in photoreceptor protection. The potential of antioxidants to slow human dystrophies requires systematic investigation, however.

6.3.1.3. *Prevention of light adaptation will slow dystrophies*

The idea that reducing the light falling on the retina might slow the progress of retinal dystrophies was proposed by Berson (1971, 1973), who traced the history of the idea in the treatment of human RP and in animal models. The present analysis builds on the rationale which Berson developed.

Light adaptation of rods increases oxygen levels in the outer retina (Linsenmeier, 1986; Brown *et al*., 1996), because rods use less oxygen when light-adapted. As long as rods persist in significant numbers, minimising their adaptation to light should minimise daily light-induced increases in oxygen levels in outer retina and thereby slow late-stage dystrophy. Dowling and Sidman (1962) reported that dark rearing slowed the late stages of the RCS dystrophy, though in their data the slowing was clear in one strain of RCS rat (pink-eye) and not in a more heavily pigmented strain. Kaitz and Auerbach (1978) revisited this problem, showing that the RCS dystrophy is markedly slowed if the wavelength and intensity of circadian light are chosen to minimise the absorption of light by rhodopsin. Naash *et al*. (1996) noted that dark rearing slows the dystrophy caused in mice by human RP transgenes and, like Kaitz and Auberbach, suggested that the slowing resulted from a decrease in the absorption of light by rhodopsin. We suggest that the reduction in light absorption by rhodopsin slows the dystrophy by maintaining the high oxygen consumption of dark-adapted rods, thereby reducing the level of oxygen in outer retina.

There are few published data which test this idea in humans. The data in Table 1 are from Merin and Pe'er (1981), who followed RP patients who wore a dark contact lens on one eye for up to 3 years, the other eye serving as a control (as Berson (1971, 1973) suggested). The lens was brown in colour and reduced total trans-

Table 1. Visual field data of RP sufferers wearing dark contact lens on one eye. Participants had a clinical diagnosis of retinitis pigmentosa (5 sporadic, 6 autosomal recessive, 3 sex-linked), a visual acuity of not less than 6/30 at commencement, and visual fields of not less than 20° (longer axis). "First" is the visual field size before the trial; "last" is the visual field size at the last of 2–7 examinations, 12–40 months later. Scotomas, where present, were subtracted from the visual field. Each participant (except one who requested a dark lens on both eyes) wore a transparent contact lens on one eye and a dark, "protective" lens on the other. The dark lens appeared brown in colour. Transmission over the visible range was least at $\lambda < 550$nm. Lenses of thickness 0.1–0.4 mm were used; at longer wavelengths their transmission varied from 8–40%, at shorter wavelengths from 45–78%. On a 2-tailed paired *t*-test comparing percent field loss in the two eyes of patients 2–14, $P < 0.02$ that they are drawn from samples of equal means.

Patient		Size of visual field (mm^2) (II/4 on Goldmann perimeter)				Field loss		Remarks
		Treated eye		Control eye				
No.	Period (mos)	First	Last	First	Last	Treated	Control	
1	40	400	360			10		Both eyes treated at patient request.
		364	364			0		Both did well.
2	35	364	364	5730	3850	18	33	Treated eye better
3	13	5000	4100	298	256	3	14	Treated eye better
4	32	2810	1720	3020	1250	39	59	Treated eye better
5	30	5430	3720	5450	3760	31.5	31	Eyes equal
6	12	464	347	445	332	25	25	Eyes equal
7	18	510	425	288	113	17	41	Treated eye better
8	18	4400	1980	2830	930	55	67	Treated eye better
9	14	765	342	553	152	55	73	Treated eye better
10	16	1553	1527	1841	1322	2	28	Treated eye better
11	17	243	141	238	141	42	41	Eyes equal
12	13	605	612	844	584	0	31	Treated eye better
13	20	2364	1079	1988	1324	54	33.5	Control eye better
14	16	1945	1200	1929	1143	38	41	Eyes equal

mission of light to 38.5% or less and, in the longer wavelengths, to as little as 10%. In 10 of the 14 patients the contraction of the visual field appeared slowed in the dark-lens eye. We are currently repeating this study on a new group of patients, to be assessed with a wider range of techniques.

6.3.2. *Therapy for the depleted retina: other approaches*

6.3.2.1. *Dietary vitamin A*

Berson and colleagues (reviewed in Berson, 1993) have reported a weak beneficial effect of high levels of dietary vitamin A on the course of human retinal dystrophies. Vitamin A is a precursor of rhodopsin and many forms of RP are caused by rhodopsin mutations, but the theoretical basis of this treatment has not been developed in more detail. Experimental work reported recently by Berson and colleagues (Li *et al.*, 1998) has begun the analysis of the mechanism of this effect, showing in a mouse model of a rhodopsin mutation dystrophy that vitamin A slows the dystrophy. Dietary supplementation of vitamin A is (at time of writing) the only form of therapy for human dystrophies to have at least the qualified support of the International Retinitis Pigmentosa Association.

6.3.2.2. *Trophic factors*

bFGF can protect photoreceptors in already depleted, presumably hyperoxic, retinas. For example, laser burns in the RCS eye protect surrounding photoreceptors for some weeks, well after the critical period (Humphrey *et al.*, 1993b) and that protection may be mediated by bFGF, which is upregulated in the protected photoreceptors (Xiao *et al.*, 1998:, Chu *et al.*, 1998). LaVail and colleagues have tested the effectiveness or a range of factors in slowing the dystro-

phies which occur in the RCS rat (LaVail *et al*., 1992a), in several mouse mutants (LaVail *et al*., 1998) and in transgenic rats made dystrophic by mutant opsin transgenes (Steinberg *et al*., 1997). They demonstrated that bFGF can slow these dystrophies over periods extending from the critical period in these strains to considerably older ages. The present analysis suggests that the late stage of dystrophy is driven by a factor (oxygen toxicity) which is chronic. This would seem to require that trophic factors be delivered chronically and to raise two problems, the effect of long term exposure to such factors on other elements of the eye, such as the lens (Chamberlain and McAvoy, 1997), and their effect on the function of the surviving retina (Section 3.4).

6.3.2.3. *Hyperbaric hyperoxia*

Several groups with access to hyperbaric facilities have tested the efficacy of hyperbaric oxygen exposure on the course of human retinal dystrophies. The most systematic study is that of Vingolo and colleagues (Vingolo *et al*., 1997) who report on 24 RP patients subjected to standard patterns of this treatment. Patients were exposed to 2.2 atmospheres of pressure for 90 min per day once a day for 5 days per week. During each session they breathed 100% oxygen through a mask. This treatment was given for a period of 4 weeks, then at decreasing intervals for up to three years. The authors reported that the ERG declined significantly less in treated patients than in a control group with comparable disease forms.

Hyperbaric hyperoxia delivers high levels of oxygen to all body tissues by increasing the amount of oxygen dissolved in serum. The increase is achieved partly by the hyperbaria, partly by the hyperoxia. It is the treatment of choice for gas gangrene and carbon monoxide poisoning and appears to have beneficial effects on, for example, the peripheral circulatory problems of diabetes (Tibbles and Edelsberg, 1996).

Hyperbaric hyperoxia is a complex treatment, involving very high levels of oxygen tension presented in daily episodes, with major changes in oxygen levels both at onset and offset of each exposure (exposure must be limited because of the toxicity of oxygen). At present these results are intriguing and invite further testing but, to the present writers, remain difficult to explain.

6.3.3. *Treatment of late stage RP will provide a limited improvement in vision*

It seems possible that the blindness of the later stages of RP stems from two sources, the loss of some photoreceptors and the loss of sensitivity in the survivors. The loss of sensitivity could result from two causes, the upregulation of factors which both protect photoreceptors and reduce the ERG in the presence of normal outer segment morphology (Section 3.4); and/or from a reduction in the photopigment content of outer segments, as described in the response of the normal retina to bright circadian light (Penn *et al*., 1989). Therapy which relieves stress on photoreceptors surviving in RP patients will presumably downregulate their self-protective mechanisms and may allow regrowth of the outer segments. Penn *et al*. (1989) described an impressive instance of such regrowth. In rats raised in bright circadian light, many photoreceptors are lost and the outer segments of the survivors are greatly shortened and their rhodopsin content is correspondingly reduced. Nevertheless, the retina is intact and functional. If these rats are returned to dim circadian light then within 3 weeks the length of their outer segments and their pigment content triple and the ERG increases markedly in amplitude (the b-wave by 60%, the a-wave by 250%).

Therapy which successfully reduced stress on photoreceptors surviving in late stage dystrophies might similarly increase their sensitivity, to a limit set by the actual loss of photoreceptors. The present analysis also suggests a cautionary conclusion. Therapy which relieves the stress on photoreceptors may also downregulate their self-protection, making it important to maintain the continuity of the treatment, lest the cells re-experience stress while unprotected.

6.3.4. *The senescence of the retina can be slowed*

The mammalian retina degenerates with age, so reliably that progressive loss of sensitivity and

acuity in advanced age is considered normal. The edge of the human retina undergoes an age-related "cystic" degeneration and systematic counts of retinal neurones (Gao and Hollyfield, 1992) show a generalised age-related loss of neurones in all layers. The rate of loss was greatest in the youngest ages studied (the teen years) but continued throughout life, slowly depleting the retina of functional neurones. Correspondingly, vision slowly fails with advanced age (Section 1.2).

The normal senescence of the retina can thus be regarded as a very slow retinal dystrophy. We have argued above that hyperoxia of the outer retina is a major factor driving the late stages of all retinal dystrophies. A logical extension of this argument is that oxygen toxicity of outer retina may be a factor in the senescence of the retina, and that treatments which slow late stage dystrophies will slow senescent changes. We are currently testing this idea in models of retinal senescence in the rat (Shinowara *et al*. (1982); Lai *et al*., 1978; DiLoreto *et al*., 1996).

These predictions extend logical argument well beyond present data. They are made to be tested, confirmed, disproved. If only partially confirmed they could provide a basis for the treatment of long—untreatable dystrophies caused by genetic mutation, by environmental insult, by age.

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