The Photoreactivity of the Retinal Age Pigment Lipofuscin*

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The presence of the age pigment lipofuscin is associated with numerous age-related diseases. In the retina lipofuscin is located within the pigment epithelium where it is exposed to high oxygen and visible light, a prime environment for the generation of reactive oxygen species. Although we, and others, have demonstrated that retinal lipofuscin is a photoinducible generator of reactive oxygen species it is unclear how this may translate into cell damage. The position of lipofuscin within the lysosome infers that irradiated lipofuscin is liable to cause oxidative damage to either the lysosomal membrane or the lysosomal enzymes. We have found that illumination of lipofuscin with visible light is capable of extragranular lipid peroxidation, enzyme inactivation, and protein oxidation. These effects, which were pH-dependent, were significantly reduced by the addition of the antioxidants, superoxide dismutase and 1,4-diazabicyclo(2,2,2)-octane, confirming a role for both the superoxide anion and singlet oxygen. We postulate that lipofuscin may compromise retinal cell function by causing loss of lysosomal integrity and that this may be a major contributory factor to the pathology associated with retinal light damage and diseases such as age-related macular degeneration.

The age pigment lipofuscin accumulates within the lysosomal system of a variety of postmitotic cells throughout life and is considered to be a biomarker of cell aging. Evidence has shown that the rate of lipofuscin accumulation corresponds to the aging rates in different species, being influenced by both metabolic activity and extent of oxidative stress (1). However, a causal role for lipofuscin in the aging process or development of age-related diseases such as neuronal ceroid lipofuscinosis, age-related macular degeneration, Menière’s disease, and cardiovascular hypertrophy has yet to be established (see Refs. 2 and 3). Unlike other cells in the body, in which lipofuscin occurs through the autophagic breakdown of intracellular organelles (2), the major substrate for lipofuscin in the retinal pigment epithelium (RPE)3 of the eye is the undegradable end product resulting from the phagocytosis of photoreceptor outer segments (4, 5) that are rich in polyunsaturated fatty acids and vitamin A.

Ocular lipofuscin may have a unique role to play in aging of the RPE, a tissue that is continually exposed to visible light (400–700 nm) and high oxygen tensions (~70 mm Hg). Studies have shown this type of lipofuscin to be a photoinducible generator of superoxide ions, singlet oxygen, hydrogen peroxide, and lipid peroxides (6–9), all of which are reactive oxygen species implicated in general aging processes. These species can adversely affect cell function by damaging proteins, carbohydrates, DNA, and lipids (10). The position of lipofuscin within the lysosome infers that the first site of oxidative damage will be either the lysosomal membrane or the lysosomal enzymes. To test this hypothesis we have assessed the effect of photoactivated lipofuscin on (i) lipid peroxidation, (ii) enzyme function, and (iii) protein oxidation. We have confirmed that lipofuscin granules incubated with visible light induce lipid peroxidation and cause enzyme inactivation supporting our hypothesis that lipofuscin contributes to aging of the RPE and is a risk factor for age-related macular degeneration.

EXPERIMENTAL PROCEDURES

Chemicals—Chemicals (at least reagent grade, unless otherwise stated) were purchased from Sigma or BDH and used as supplied. Acid phosphatase, catalase (thymol-free), and superoxide dismutase were purchased from Sigma. Universal buffer contained 30 mM citric acid, KH2PO4, and boric acid and was adjusted to the appropriate pH with 0.2 M NaOH.

Isolation of Lipofuscin—Lipofuscin granules were isolated from 60–70-year old individuals according to a procedure previously described (11) except that mechanical homogenization was used instead of ultrasonication. Isolated granules were resuspended in 10 mM phosphate buffer, pH 7.2, and dispersed by forcing them through a narrow gauge needle. Concentration of the granules was determined by counting on a hemocytometer. Solutions were diluted according to the methods described below.

Light Exposure—the reaction mixtures, prepared in 7-ml glass vials were incubated for up to 8 h in the presence or absence of full white light (400–1100 nm, 80–90 milliwatts/cm²) from a 15-V/180-watt halogen-ellipsoid-reflektorlampe KL 1500 (Schott, Mainz, Germany) transmitted through glass fiber optics as described previously (6). The samples were constantly agitated using a magnetic stirrer. Illumination did not cause an increase in temperature during the incubation period, and each experiment was performed at least three times.

Preparation of Substrates for Lipid Oxidation—Confluent human RPE cultures (passage 4) (12) were trypsinized and resuspended at 1 × 10⁶ cells/ml in universal buffer, pH 7.0. Bovine photoreceptor outer segments (POS) were isolated by the method of Papermaster (13) and resuspended at a concentration of 1 × 10⁷ POS/ml in universal buffer, pH 7.0. Samples were either used immediately or aliquoted (1 ml) for storage at ~20 °C under nitrogen until use.

Lipid Oxidation—the substrates (2.5 ml), with or without lipofuscin (final concentration, 1 × 10⁷ granules/ml) were incubated for up to 4 h in the presence or absence of white light. At 0-, 2-, and 4-h time intervals duplicate 0.4-ml samples were removed and analyzed for lipid oxidation by the thiobarbituric acid (TBA) assay as described by Buege and Aust (14). Substrate incubated with 25 μl of ascorbic acid (2.5 mM)/50 μl of Fe²⁺ sulfate (2.5 mM) was used as a positive control. A negative control was prepared by replacing substrate with 2.5 ml of...
universal buffer. The experiments were repeated in the presence of the antioxidants superoxide dismutase (SOD; final concentration, 30 μg/ml) (6) or 1,4-diazabicyclo(2.2.2)octane (DABCO; final concentration, 30 μM) (15).

**The Effect of pH on Lipofuscin-induced Lipid Oxidation**—The generation of certain reactive oxygen species and their ability to induce oxidation is pH-dependent (16). Therefore, the pH dependence of lipofuscin photoreactivity was determined. To allow comparison between the different pH values, the same batch of POS was used. In brief, suspensions of POS at 1 × 10⁷/ml in universal buffer at pH 4.5, 7.0, and 10 were prepared. The amount of oxidation occurring in samples incubated with and without lipofuscin in the presence and absence of light over a 90-min period was determined by the TBA assay using duplicate 0.4-ml samples at time 0, 30, 60, and 90 min.

**Effect of Lipofuscin on Catalase Activity**—5 ml of catalase (300 units/ml phosphate buffer, pH 7.4) together with either 20 μl of lipofuscin (final concentration, 1 × 10⁷ granules/ml) or phosphate-buffered saline were incubated in the presence or absence of white light for up to 1 h. At 15-min intervals, 900-μl aliquots were taken and immediately centrifuged at 15,000 × g for 3 min. 800 μl of the resulting supernatant was stored at 4 °C until the activity of catalase was assayed by the method of Cohen et al. (17). Experiments were repeated in the presence of SOD and DABCO as described above and mannitol (final concentration, 24 mM).

**The Effect of Lipofuscin on Acid Phosphatase Activity**—The effect of lipofuscin on acid phosphatase activity was determined at pH 4.5 and 7.0 (enzyme inactivation occurred at pH > 7.0). 1 ml of acid phosphatase (10 milliunits/ml) was incubated for 1 h both with (1 × 10⁷ granules/ml) and without lipofuscin at room temperature in the presence or absence of white light. At 15-min intervals, 125-μl aliquots were taken and immediately centrifuged at 15,000 × g for 3 min to pellet the lipofuscin. 100 μl of the supernatant was then taken and stored at −20 °C prior to analysis of enzyme activity using an assay based on the microassay of Cabral et al. (18). Experiments were repeated in the presence of SOD and DABCO as described above.

**The Effect of Lipofuscin on Protein Oxidation**—2.5 ml of bovine serum albumin (BSA) was prepared at 100 μg/ml in universal buffer, pH 4.5 and 7.0, with (final concentration, 4 × 10⁷ granules/ml) or without lipofuscin. The samples were maintained at room temperature in the dark or exposed to white light for 8 h. 1-ml aliquots were removed at time 0 and after 8 h and centrifuged at 15,000 × g for 3 min to pellet the lipofuscin. 2 × 450-μl aliquots were stored under nitrogen at −20 °C prior to analysis for 1) tryptophan oxidation and 2) protein aggregation/fragmentation.

**Tryptophan Fluorescence**—A measure of tryptophan oxidation, was determined by fluorescence spectroscopy with excitation at 285 nm and emission at 345 nm (19). Protein aggregation/fragmentation was determined by SDS-polyacrylamide gel electrophoresis. In brief, reduced and non-reduced samples were loading onto a stacking polyacrylamide gel (12% running gel and 3% stacking gel). Analysis of band intensity, following staining with 0.1% w/v Coomassie blue, was determined using an Ultrascan XL enhanced laser densitometer (Amersham Pharmacia Biotech), taking triplicate readings per band.

**Statistical Analysis**—Each experiment was performed at least three times. Rates of lipid oxidation were estimated by fitting a linear model to the data using the program Intrate in the Simfit statistical package (20) after normalization of the data at time 0.

To allow for interexperimental variations due to enzyme and lipofuscin, batch enzyme activity is expressed as a decrease relative to time 0 rather than an absolute value. Rates of enzyme inactivation were estimated by fitting an exponential model to the decay data after normalization of the data at time 0 using the program QNPFIT in the Simfit package, which performs constrained non-linear least squares regression (20). Under “Results,” inactivation rates are quoted as change in absorbance units (AU) per min ± 95% confidence limits and were calculated from the mean of values obtained from at least three independent experiments.

**RESULTS**

**The Effect of Lipofuscin on Lipid Oxidation**—Photoxidation of cell membranes and POS was greatest in the presence of lipofuscin and increased with increasing duration of light exposure (Fig. 1). After 4 h of incubation the irradiated samples containing lipofuscin demonstrated 2.3 and 1.8 times more TBA-reactive products for cell membranes and POS, respectively, compared with irradiated samples in the absence of lipofuscin (p < 0.01). Lipid substrates exposed to light alone demonstrated significant oxidation, but this was always less than in the presence of lipofuscin. There was no significant increase in TBA-reactive products in samples (both with and without lipofuscin) maintained in the dark or samples with lipofuscin alone exposed to light throughout the 4-h incubation. Although the same trend was seen in all experiments overall values varied between batches of lipofuscin and POS. Further experiments were undertaken using POS as substrate because of their greater susceptibility to lipid peroxidation. The degree of lipid peroxidation was pH-dependent (data not shown); oxidation of POS was observed in the presence of lipofuscin and light at pH 7.0 but was not detectable at either pH 4.5 or 10.

The addition of the antioxidants SOD and DABCO resulted in a significant reduction in the rate of lipofuscin-photoinduced lipid peroxidation (p < 0.05) (Table I). POS incubated with lipofuscin and light demonstrated a mean oxidation rate of 20.5 × 10⁻⁸ AU/min compared with 7.9 and 8.0 × 10⁻⁸ AU/min in the presence of SOD and DABCO, respectively. Light-induced oxidation of POS in the absence of lipofuscin was also significantly reduced in the presence of DABCO and SOD.

**The Effect of Lipofuscin on Enzyme Activity**—Catalase incubated in the presence of light and lipofuscin was inactivated at a significantly higher rate than controls incubated under identical conditions but without lipofuscin (p < 0.05); samples incubated with lipofuscin exhibited approximately 2.5 times more activity than controls at the 60-min time point (Fig. 2). In the absence of light, catalase inactivation occurred more slowly (p < 0.05), and no significant differences in inactivation rates between samples incubated with and without lipofuscin were observed. Because lipofuscin affected catalase activity only in the presence of light the effect of antioxidants on the inactivation process was determined only under such conditions (Table II). The addition of SOD failed to protect against lipofuscin-induced catalase inactivation suggesting that superoxide ions were not involved. Inclusion of DABCO in the incubation mixture resulted in a significant decrease in the rate of lipofuscin-pho-toinduced catalase inactivation (p < 0.05) with samples incubated with both lipofuscin and DABCO demonstrating a mean inactivation rate of 1.5 × 10⁻⁸ AU/min compared with 22 × 10⁻⁸ AU/min for samples incubated with lipofuscin plus light. In the absence of lipofuscin DABCO itself had no significant effect on the rate of catalase inactivation. The addition of mannitol, a scavenger of hydroxyl radicals, to the incubation mixture had no significant effect on the rate of lipofuscin-induced catalase inactivation or the assay system.

In contrast to the light-dependent effect of lipofuscin on catalase, lipofuscin reduced acid phosphatase activity in both the presence of SOD and DABCO as described above and mannitol (final concentration, 24 mM).
The effect of antioxidants on lipofuscin-induced lipid oxidation of photoreceptor outer segments (Table I). POS (1 × 10^7/ml) were incubated in the presence and absence of lipofuscin (final concentration, 1 × 10^7 granules/ml), with and without white light for 4 h. Experiments were undertaken in the presence or absence of SOD (final concentration, 30 μg/ml) or DABCO (final concentration, 30 mM). At 0, 2-, and 4-h time intervals samples were removed and analyzed for lipid oxidation by the TBA assay. Lipid oxidation was determined by monitoring the formation of TBA-reactive products at 532 nm using the TBA assay.

<table>
<thead>
<tr>
<th>Incubation conditions</th>
<th>Mean oxidation rate × 10^-4 (mean ± 95% confidence limits)</th>
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<tbody>
<tr>
<td>+ Light</td>
<td>12.6 (8.5–16.7)</td>
</tr>
<tr>
<td>+ Light + SOD</td>
<td>7.6 (5.7–9.6)</td>
</tr>
<tr>
<td>+ Light + DABCO</td>
<td>7.4 (4.2–9.3)</td>
</tr>
<tr>
<td>Lipofuscin + light</td>
<td>20.5 (20–21)</td>
</tr>
<tr>
<td>Lipofuscin + light + SOD</td>
<td>7.9 (7.1–8.8)</td>
</tr>
<tr>
<td>Lipofuscin + light + DABCO</td>
<td>8 (7.3–8.7)</td>
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The effect of antioxidants on lipofuscin-induced inactivation of catalase (Table II). Catalase (300 units/ml in phosphate-buffered saline, pH 7.4) was incubated in the presence and absence of lipofuscin (final concentration, 1 × 10^7 granules/ml), with and without white light for up to 60 min at pH 7. Experiments were performed in the presence or absence of SOD (final concentration, 30 μg/ml), DABCO (final concentration, 30 mM), or mannitol (final concentration, 24 mM). Samples were removed at 15-min intervals, and catalase activity was determined by monitoring the rate of H_2O_2 (50 μM) decomposition at 240 nm, 15 and 30 s after addition of enzyme. To allow for interexperimental variations due to enzyme and lipofuscin, batch enzyme activity is expressed as a decrease relative to time 0 rather than an absolute value.

<table>
<thead>
<tr>
<th>Incubation conditions</th>
<th>Mean inactivation rate × 10^-3 (mean ± 95% confidence limits)</th>
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<tbody>
<tr>
<td>+ Lipofuscin + light</td>
<td>22 (20–24)</td>
</tr>
<tr>
<td>+ Lipofuscin + light + SOD</td>
<td>17 (14–20)</td>
</tr>
<tr>
<td>+ Lipofuscin + light + DABCO</td>
<td>12 (10–14)</td>
</tr>
<tr>
<td>+ Lipofuscin + light + mannitol</td>
<td>22 (15–29)</td>
</tr>
<tr>
<td>+ Light</td>
<td>8.2 (6.4–10)</td>
</tr>
<tr>
<td>+ Light + SOD</td>
<td>7.5 (6.8–8.3)</td>
</tr>
<tr>
<td>+ Light + DABCO</td>
<td>7.6 (6.5–9.8)</td>
</tr>
<tr>
<td>+ Light + mannitol</td>
<td>8.7 (7.1–10.3)</td>
</tr>
</tbody>
</table>

FIG. 2. The effect of lipofuscin on catalase activity. Catalase (300 units/ml in phosphate-buffered saline, pH 7.4) was incubated in the presence (■) and absence (▲) of lipofuscin (final concentration, 1 × 10^7 granules/ml), with (dashed lines) and without (continuous lines) white light for up to 60 min at pH 7.0. Samples were removed at 15-min intervals, and catalase activity was determined by monitoring the rate of H_2O_2 (50 μM) decomposition at 240 nm, 15 and 30 s after addition of enzyme. To allow for interexperimental variations due to enzyme and lipofuscin, batch enzyme activity is expressed as a decrease relative to time 0 rather than an absolute value. Results are for a typical experiment and are shown ± S.E.

The Photoreactivity of Lipofuscin (Fig. 3). Acid phosphatase activity in samples incubated with lipofuscin were inactivated at a significantly higher rate (~2.5 × 10^{-2} AU/min) than controls (~1.4 × 10^{-2} AU/min) incubated in the absence of lipofuscin (p < 0.05) (Table III). The rate of lipofuscin-induced enzyme inactivation in the presence of light was significantly reduced from ~2.5 × 10^{-2} AU/min to ~1.4 × 10^{-2} AU/min by inclusion of SOD in the incubation mixture (p < 0.05) (Table III). Inclusion of SOD in the absence of light reduced the effect of lipofuscin from 2.6 × 10^{-2} AU/min to ~1.8 × 10^{-2} AU/min. In the absence of lipofuscin and light, SOD had no effect on enzyme inactivation rates or the amount of residual activity after 60 min of incubation. DABCO was not assessed because it appeared to interfere with the assay system in the presence of light. When the above experiments were repeated at pH 4.5, no significant differences in inactivation rates between samples incubated with and without lipofuscin in the presence or absence of light were found.

The Effect of Lipofuscin on Protein Oxidation—In the presence of light, lipofuscin induced a small (13.6 ± 2.0%) but significant reduction in tryptophan fluorescence after 8 h of incubation at pH 7.0 compared with light alone or in the dark in the presence or absence of lipofuscin (Fig. 4). No significant effects were seen after incubation at pH 4.5. If oxidation had caused aggregation or fragmentation of the BSA a change in the single BSA band would have been observed. However, lipofuscin in the presence and absence of light failed to demonstrate any differences in either the density of the major BSA band or the appearance of additional higher or lower molecular weight bands (data not shown).

FIG. 3. Comparison of the effects of lipofuscin on acid phosphatase activity. Acid phosphatase (10 milliunits/ml) was incubated in the presence (■) and absence (▲) of lipofuscin (final concentration, 1 × 10^7 granules/ml), with (dashed lines) and without (continuous lines) white light for up to 60 min at pH 7.0. Samples were removed at 15-min intervals, and acid phosphatase activity was determined by monitoring p-nitrophenyl phosphate (5 mM) degradation at 405 nm. To allow for interexperimental variations due to enzyme and lipofuscin, batch enzyme activity is expressed as a decrease relative to time 0 rather than an absolute value. Results are for a typical experiment and are shown ± S.E.

DISCUSSION

This study has shown that lipofuscin can photoinduce the oxidation of lipid membranes and inactivate enzymes and that such effects are mediated by the production of reactive oxygen species. Age-related damage to lipid membranes and cellular proteins by such species has been implicated in the general aging process (reviewed in Ref. 21). This is the first demonstration that lipofuscin-derived reactive oxygen species can induce such effects. Although the light intensity used in this study is ~100-fold greater than normal retinal irradiance it is at least
with lipofuscin; of white light at pH 7.0. Samples were taken after 0 and 8 h of incubation and assayed for tryptophan fluorescence by fluorescence spectroscopy with excitation at 285 nm and emission at 345 nm. Results are expressed as a decrease relative to time 0 rather than an absolute value.

Table III

<table>
<thead>
<tr>
<th>Incubation conditions</th>
<th>Mean inactivation rate ( \times 10^{-2} )</th>
<th>Range ( \times 10^{-2} ) (mean ( \pm ) 95% confidence limits)</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Light</td>
<td>1.2</td>
<td>0.9–1.5</td>
</tr>
<tr>
<td>+ Light</td>
<td>1.5</td>
<td>1.0–1.8</td>
</tr>
<tr>
<td>+ Lipofuscin - light</td>
<td>2.6</td>
<td>2.3–3.0</td>
</tr>
<tr>
<td>+ Lipofuscin + light</td>
<td>2.5</td>
<td>2.0–3.0</td>
</tr>
<tr>
<td>+ Lipofuscin - light + SOD</td>
<td>1.8</td>
<td>1.0–2.6</td>
</tr>
<tr>
<td>+ Lipofuscin + light + SOD</td>
<td>1.4</td>
<td>0.9–1.9</td>
</tr>
</tbody>
</table>

**Fig. 4. The effect of lipofuscin on BSA tryptophan fluorescence.** BSA (100 μg/ml) was incubated with and without lipofuscin (final concentration, 1 \( \times 10^5 \) granules/ml) in the presence and absence of white light at pH 7.0. Samples were taken after 0 and 8 h of incubation and assayed for tryptophan fluorescence by fluorescence spectroscopy with excitation at 285 nm and emission at 345 nm. Results are expressed as a decrease relative to time 0 rather than an absolute value.

100-fold less than that achieved when sun gazing. Thus the light intensity used in this study equates to that which induces photochemical-induced retinal cell loss in animals.

With lipid oxidation, lipofuscin-derived superoxide ions appeared to be more important than singlet oxygen; inclusion of SOD in the incubation mixture induced a greater reduction in the effect of lipofuscin compared with the singlet oxygen scavenger DABCO. This result is surprising, because the redox potential of the \( \text{O}_2^*/\text{O}_2 \) couple at \(-330 \text{ mV}\) means that it is incapable of removing an alliconic hydrogen from polyunsaturated fatty acids (PUFA) during the oxidation process. PUFA/H - PUFA-H = 600 mV, whereas singlet oxygen is a sufficiently powerful oxidizing agent to exert such an effect (O(1Δg)/O2 = 650 mV). At acidic pH values, the protonated form of superoxide (HOO2+) is a sufficiently strong oxidizing agent to carry out PUFA oxidation. However, lipofuscin only exerted its effect at neutral pH, suggesting the involvement of some other mechanism. Superoxide ions can give rise to the highly reactive hydroxyl radical via the Haber-Weiss reaction; this radical is capable of inducing lipid oxidation. The singlet oxygen may have been formed from lipofuscin itself, superoxide dismutation, interaction between superoxide and the hydroxy radical, or disproportionation of the lipid peroxy radical. It appeared to be more important toward the latter stages of the experiment (i.e. DABCO afforded a 39% protection at 2 h, rising to 50% after 4 h). The lag phase may be necessary to allow the generation of other products needed for singlet oxygen formation or damage to the granule membrane to allow these species to escape to exert their effects extragranularly.

Concerning the effect of lipofuscin and light on enzyme function, the amount of inhibition and importance of individual reactive oxygen species depended on the type of enzyme. A greater effect was obtained when catalase rather than acid phosphatase was used. This was probably as a consequence of the heme groups contained within the catalase structure at the enzyme’s active site (24, 25). It was interesting that lipofuscin had a significant effect on acid phosphatase activity in the absence of light and suggests that in this case, two independent mechanisms were responsible for the inhibition of enzyme function. For catalase, singlet oxygen was the key reactive species in inducing damage, with no significant role of either superoxide or the hydroxy radical. However in this case, the involvement of superoxide ions was difficult to assess because of the spontaneous dismutation of these ions by catalase. These different susceptibilities to damage were probably reflections of the types, accessibility, and importance of oxidizable residues within the protein backbone and active site of the individual enzymes (26). Gantchev and van Lier (27) found catalase to be inactivated by singlet oxygen. However, in their study the hydroxy radical was also implicated, and oxidative damage to the protein aggregates. These differences were probably due to 1) the free radical generating system used in their study; their system was soluble, possibly allowing generation of reactive oxygen species closer to the target and 2) the more drastic oxidizing conditions (i.e. long exposure periods and higher light exposures). Our study also demonstrated that lipofuscin is capable of oxidizing tryptophan within BSA. Using different regenerating systems Ogino and Okada (28) and Miura et al. (29) also reported a free radical-induced decrease in tryptophan fluorescence. They also reported BSA fragmentation to be associated with free radical damage. No such effect was observed in this study; this may reflect the different free radical generating system.

Whether lipofuscin can exert these oxidative effects in vivo remains open to question. No studies have examined age-related changes in the oxidative state of the RPE cell membrane, although evidence exists for age-related increases in the susceptibility to such damage of both RPE cell membranes (30) and membranes from other cell types (31–34). These changes may result in decreased membrane fluidity (31) and increased membrane permeability (35). If such changes are induced by lipofuscin in vivo, they are likely to initially occur at the level of the lysosomal membrane. Evidence for this is provided by Wihlmark et al. (36), who demonstrated that the accumulation of lipofuscin-like material causes lysosomal instability following irradiation with blue light. Alternatively, damage to membrane potential due to damage of the proton pump may decrease the hydrogen ion concentration of the lysosome and hence alter enzyme function. The pH dependence of lipofuscin-induced effects suggests that reactive oxygen species pass part way through the lysosomal membrane, exerting their effects in the local environment of the lipid tail groups that will be at a more physiological pH. Age-related variations in RPE enzyme activities have been investigated and found to increase with donor age (37). In other cell types, such changes have been associated with higher levels of lipofuscin (38) and may be an attempt by the cell to digest this pigment or to increase enzyme activity in response to lipofuscin-induced enzyme inactivation. The next stage in demonstrating a direct role for lipofuscin in RPE cell toxicity will be to carry out intracellular studies.
REFERENCES
