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Changes in the Expression of Specific Müller Cell Proteins during Long-Term Retinal Detachment

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Retinal detachments were produced in domestic cats by injecting fluid between the retinal pigment epithelium and neural retina. Retinas were allowed to remain detached for 30 or 60 days at which time the animals were killed. Tissue areas from detached and attached retinal regions from the same eye were processed for correlative biochemical and structural analysis, i.e. SDS–PAGE and Western blots of tissue homogenates were correlated with tissue processed for postembedding immunoelectron microscopy. Antibodies to six proteins were used as probes. Glial fibrillary acidic protein in Müller cells has previously been shown to increase after retinal detachment; here we show that vimentin, another intermediate filament protein present in Müller cells, also increases after detachment. In contrast, cellular retinaldehyde binding protein, cellular retinol binding protein, glutamine synthetase, and carbonic anhydrase C—all normally found in Müller cells—decrease after detachment. The results of this study indicate that retinal Müller cells dramatically alter their expression of proteins in response to retinal detachment.

Key words: Müller cells; retina; retinal detachment; glial fibrillary acidic protein; vimentin; cellular retinaldehyde binding protein; cellular retinol binding protein; carbonic anhydrase C; glutamine synthetase.

1. Introduction

A number of morphological changes occur in the neural retina when it is experimentally separated from the retinal pigment epithelium (RPE; Kroll and Machemer, 1968; Anderson, Stern, Fisher, Erickson and Borgula, 1981; Erickson, Fisher, Anderson, Stern and Borgula, 1983). Photoreceptor outer segments rapidly degenerate to the point where only a few disorganized discs remain attached to the cilium; degeneration of cell processes, cell bodies and synaptic terminals occurs in outer nuclear (ONL) and outer plexiform layers (OPL; Erickson et al., 1983). After production of an experimental retinal detachment, there is also proliferation and migration of several cell types. Among the most reactive cells are the Müller cells, the specialized radial glia that extend the width of the retina from the outer to inner limiting membranes. By two days after detachment, Müller cell nuclei label with [^{3}H]thymidine (Erickson et al., 1983) and can be seen migrating into the OPL and ONL (Erickson et al., 1983; Anderson, Guérin, Erickson, Stern and Fisher, 1986; Erickson, Fisher, Guérin, Anderson and Kaska, 1987). At this same time, Müller cells greatly elevate their level of RNA synthesis (Erickson, Fisher, Guérin, Anderson, and Kaska, 1985) and show a build-up of intermediate filaments (Erickson et al., 1983, 1987). As the detachment time increases, the Müller cell processes expand within the OPL and ONL and sometimes extend into the subretinal space where they can interfere with photoreceptor outer segment regeneration after retinal reattachment (Erickson et al., 1983; Anderson et al., 1986a). Antibodies to specific Müller cell

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proteins were therefore used as probes to further delineate cellular and molecular changes that occur during a period of retinal degeneration induced by experimental detachment. We have previously reported the localization of these antigens in normal, unoperated cat retina (Lewis, Erickson, Kaska and Fisher, 1988). Because the detachment does not involve the entire retina, areas of attached retina from the eye with the detachment were compared to detached retinal areas.

The structural changes in the retina after detachment resemble those present in a variety of human and animal retinal disorders including: retinitis pigmentosa (Rayborn et al., 1985), macular disease (Eagle, 1984), diabetic retinopathy (Nork, Wallow, Sramek and Anderson, 1987), progressive rod-cone degeneration (Aguirre, Stramm and O'Brien, 1985), various retinal dystrophies in rats and mice (Dowling and Sidman, 1962; La Vail, 1981; Eisenfeld, Bunt-Milam and Sarthy, 1984; Sarthy and Fu, 1988), responses to penetrating ocular injuries (Bignami and Dahl, 1979; Miller and Oberdorfer, 1981) and damage caused by constant light (Eisenfeld et al., 1984; Sarthy and Fu, 1988). Therefore, the changes described in this study are likely to be part of the retina's characteristic response to acquired or inherited degenerative diseases.

2. Materials and Methods

Cat retinas were experimentally detached from the retinal pigment epithelium for 30 ($N = 2$) or 60 ($N = 2$) days. The cats were entrained to a 12:12 light cycle and were killed 4 hr after lights on, at which time several different areas from detached and attached retinas were processed for immunocytochemistry, SDS–PAGE and Western blot analysis. The procedure for producing the detachment has previously been published (Anderson et al., 1986a). Briefly, the lens was removed from the eye and the eye was allowed to heal for a minimum of 2 weeks at which time the vitreous was removed and a micropipet attached to an infusion pump was inserted between the neural retina and the RPE. The forces promoting retinal reattachment in the cat are strong enough such that in retinas with small tears, reattachment occurs spontaneously after about 7 days. In order to investigate the long-term detachments (without producing large retinal tears) we injected a 0·25% solution of Healon (sodium hyaluronate, Pharmacia) into the subretinal space through the micropipet; 30 and 60 days were chosen as possible end-points to some of the changes that occur with retinal detachment and to allow time for the formation of a subretinal scar.

Tissue for immunocytochemistry was cut into quadrants and fixed for 1 hr with 1% paraformaldehyde and 1% gluteraldehyde in sodium phosphate buffer. The retinas were then dehydrated in increasing concentrations of methanol and embedded in LR White resin (Polysciences). Uranyl acetate (2%) was added, en bloc, to 70% methanol during the dehydration as a secondary fixative to enhance contrast and to improve tissue preservation and immunolabeling of proteins (Erickson, Anderson and Fisher, 1987).

Rabbit anti-bovine-glial fibrillary acidic protein (GFAP, DAKO Corp.) and mouse anti-porcine-vimentin (DAKO Corp.) were used at a dilution of 1:400. Rabbit anti-bovine-cellular retinol binding protein (CRBP) and cellular retinaldehyde binding protein (CRALBP; both gifts from John Saari) were used at a 1:200 and 1:400 dilution, respectively. Rabbit anti-chicken-glutamine synthetase (GS) and carbonic anhydrase C (CAC; both gifts from Paul Linser) were used at a dilution of 1:600. All antibodies were diluted with phosphate buffered saline (PBS) containing 0·5% bovine serum albumin (BSA; Sigma). Normal goat serum (NGS; Vector Labs; diluted 1:75 with PBS) was used as a blocking agent on each section before applying the primary antibodies. Goat anti-rabbit (GAR) or goat anti-mouse (GAM) IgG conjugated to 5 nm gold spheres (Janssen) was used at a 1:20 dilution for light microscopic immunocytochemistry (LM ICC). The 5 nm gold was amplified using a silver-enhancement technique (Janssen). Goat anti-rabbit IgG conjugated to 15 nm gold spheres was used at a dilution of 1:20 for single-label electron microscopic immunocytochemistry (EM ICC). For double-label experiments combinations of 5 nm and

15 nm or 15 nm and 30 nm gold spheres were used as secondary antibody tags, both at a 1:20 dilution.

Double-label immunocytochemistry with anti-GFAP and anti-vimentin has previously been described (Erickson and Fisher, 1988). Briefly the primary antibodies were combined and the mixture was added to the sections. Secondary antibodies were also combined and added to the sections. Since anti-GFAP was raised in a rabbit and anti-vimentin was produced using mice, GAR and GAM secondary antibodies, conjugated to differently sized gold spheres, were used to distinguish between the two primary antibodies.

Light microscopic immunocytochemistry was performed at room temperature using 1 μm thick sections taken from detached and attached retinas from the eye with a detachment. The sections, which had been placed on formvar-coated glass slides to prevent them from being washed off the glass surface, were then covered with NGS for 30 min, blotted, and incubated overnight with the primary antibody. The following day, sections were rinsed with PBS–BSA and the secondary antibody was added for 1 hr. Sections were subsequently rinsed with PBS, and the gold spheres were enlarged using the silver enhancing procedure. When the enhancing was complete, the sections were rinsed in PBS and distilled water and air dried. Sections of attached and detached retinas for each antibody were always processed together and enhanced for the same time period to ensure equal treatment.

Thin sections for EM ICC were cut from the same blocks of tissue that were used for LM ICC. The sections were first placed on nickel grids, incubated on drops of NGS for 30 min, blotted and incubated overnight on the primary antibody. The next day the grids were rinsed with PBS–BSA and transferred to drops of the secondary antibody for 1 hr. After rinsing with PBS–BSA and distilled water, the grids were stained with uranyl acetate for 15 min and lead citrate for 7 min, exposed to osmium tetroxide vapors (2% osmium in PBS) for 1 hr, and carbon coated in a vacuum evaporator.

Normal, detached and attached retinal tissue used for SDS–PAGE and Western analysis was homogenized in 0·02 M PBS containing 0·1 mM phenylmethylsulfonylfluoride, 10 mM EDTA, 0·5% Triton X-100 and 2·55% SDS to inhibit protein degradation. Protein concentrations were determined by the BCA Protein Assay (Pierce Chemical Co.). A 50 μg sample of protein was analyzed by SDS–PAGE using a 7·5–20% gradient gel. Proteins of known molecular weights (Bio-Rad) were used as standards. Western blots were prepared by transferring the separated retinal proteins to nitrocellulose paper, blocking with 3% BSA in Tris buffered saline (TBS) and incubating overnight in primary antibody (diluted 1:100 in 1% BSA in TBS). The next day the blots were washed in Tween-Tris buffered saline (TTBS; 0·05% Tween 20) and incubated with horseradish peroxidase (HRP) labeled goat anti-rabbit or goat anti-mouse IgG (Bio-Rad) for 1 hr. Blots were again washed in TTBS and then TBS and placed in HRP Color Development Reagent (Bio-Rad) for 15 min. After distilled water rinse, the immunoblots were air dried. In order to allow comparisons of proteins from attached, detached and normal retinas, identical amounts of protein were run in adjacent gel lanes and transferred to a single piece of nitrocellulose for simultaneous immunoblotting.

3. Results

Immunocytochemical labeling of attached retinal regions with antibodies to GFAP [Figs 1(A), 2(A)] and vimentin [Fig. 2(B)] occurred predominantly over the Müller cell endfeet. Only occasionally did labeling extend beyond the inner plexiform layer. There was, however, a dramatic increase in the labeling of the Müller cells within the region of detachment. In both the 30- and 60-day detachments, anti-GFAP [Figs 1(B) and 2(C)] and anti-vimentin [Fig. 2(D)] labeling were present throughout the Müller cell cytoplasm, in some instances extending from the internal limiting membrane into the sub-retinal space between the photoreceptors and the RPE. Results from detachments were taken from the superior retina (which is thicker than the inferior retina) while the attached regions routinely came from inferior retina. This and the fact that detachment tends to produce some retinal thickening results in the retina appearing wider in sections from the area of detachment.

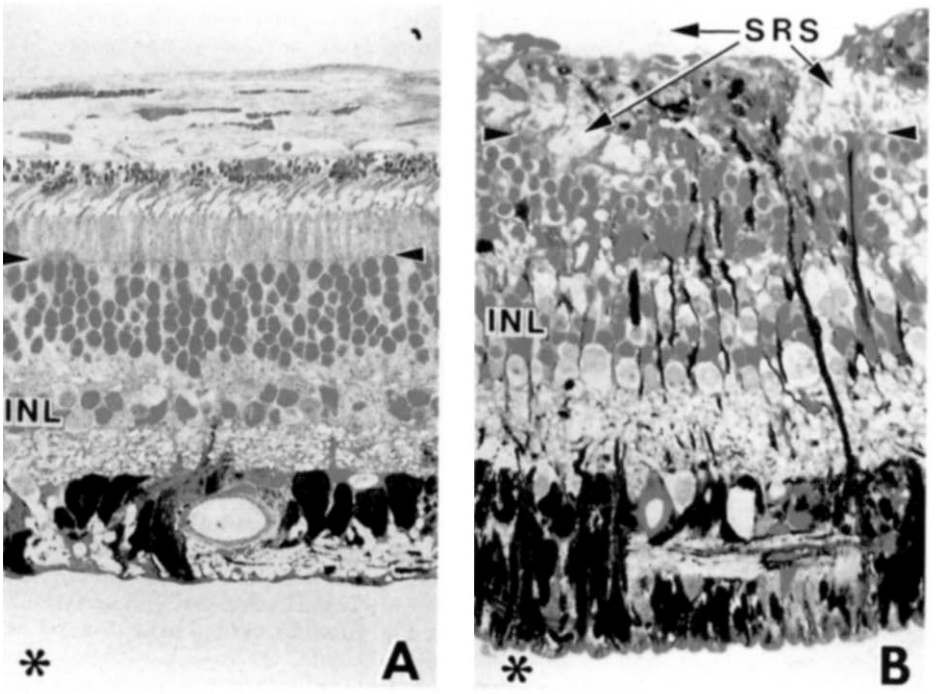

FIG. 1. Light microscopic silver enhanced immunocytochemistry using anti-GFAP on sections of attached (A) and detached retinas (B). In the attached retina, labeling is present predominantly over the Müller cell endfeet. After 60 days of detachment labeled Müller cell processes extend beyond the outer limiting membrane (arrowheads) into the subretinal space (SRS). The antibody labeling reaction product appears black against the red background of basic fuchsin counterstain. These figures can be used to provide orientation in the remaining figures showing results from sections that were not counterstained. (* vitreous cavity; INL, inner nuclear layer.) ×275.

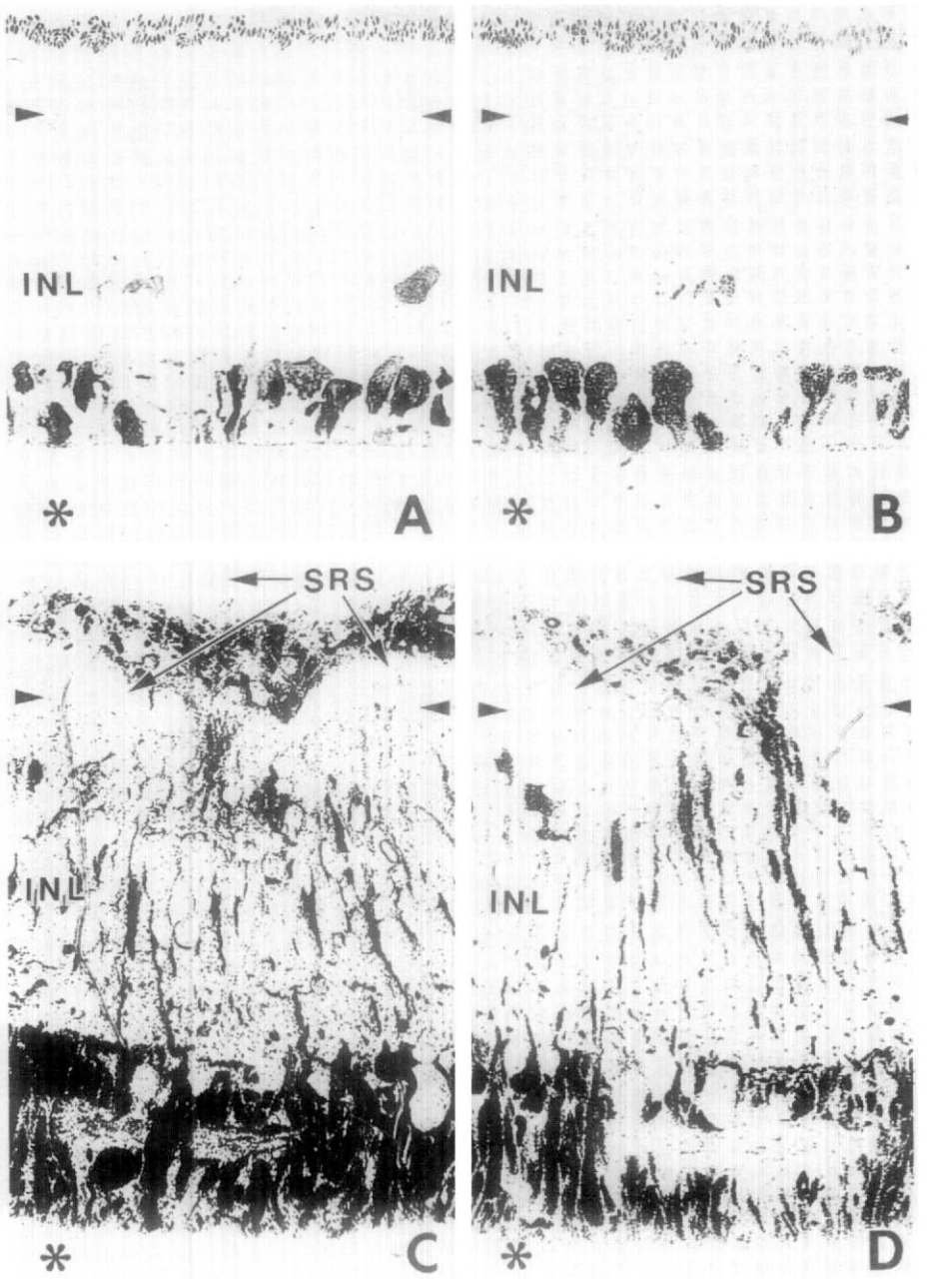

FIG. 2. Light microscopic silver enhanced immunocytochemistry using anti-GFAP (A, C) and anti-vimentin (B, D) on sections of 60 day attached (A, B) and detached (C, D) retinas. The sections are serial to those in Fig. 1 but are unstained to allow the fine detail of the labeling pattern to be resolved. Anti-GFAP and anti-vimentin labeling is greatest over the Müller cell endfeet in attached retinas (A, B) but extends into the subretinal space (SRS) in detached retinas (C, D). (The RPE and choroid in (A) and (B) contains pigment granules and no labeling.) (* vitreous cavity; INL, inner nuclear layer; arrowheads, OLM.) ×275.

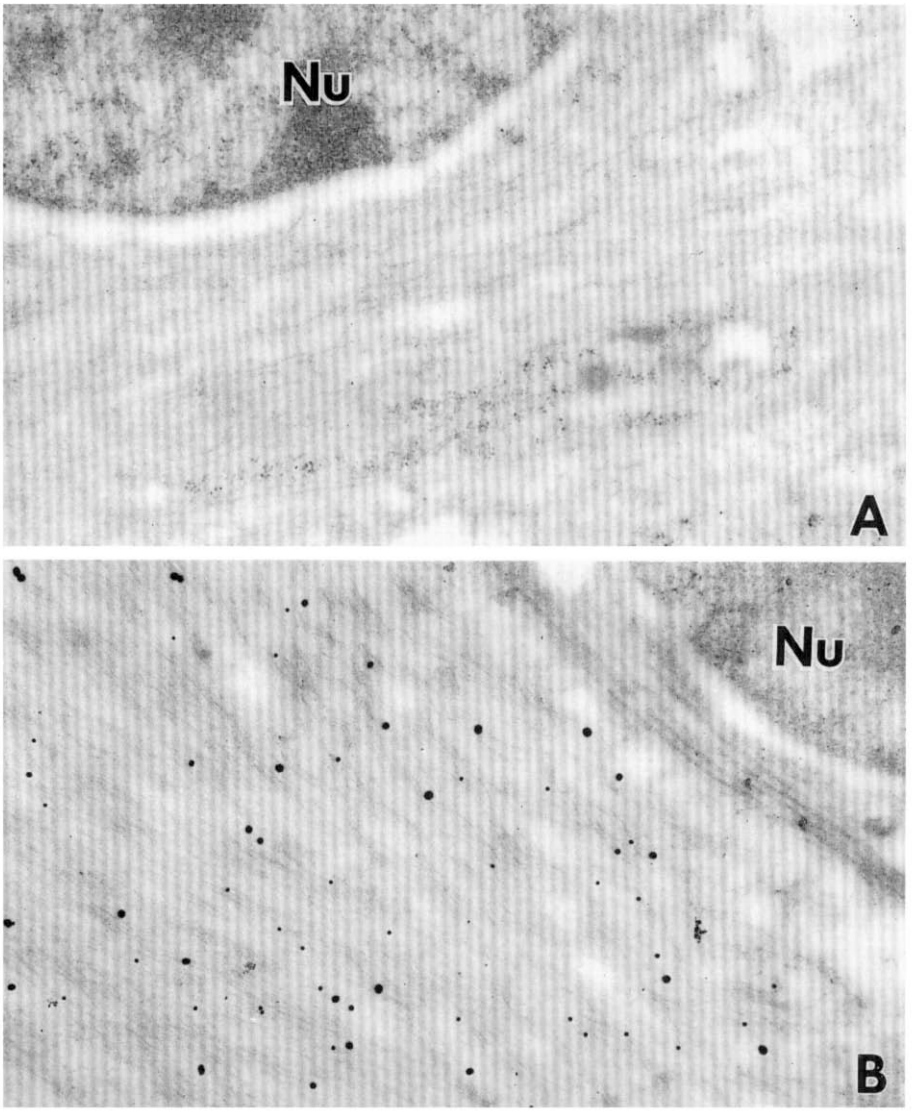

FIG. 3. Double-label electron microscopic immunocytochemistry using anti-GFAP (15 nm gold particles) and anti-vimentin (30 nm gold particles) in Müller cell cytoplasm in the region of the outer nuclear layer of 60 day attached (A) and detached retinas (B). Label, indicating the presence of 10 nm diameter filaments in Müller cell processes, is only present in the detached retina (B). (Nu, photoreceptor nucleus.) ×3100.

Using a double-label electron microscopic technique, labeling with anti-GFAP and anti-vimentin appeared over the clusters of intermediate (10 nm diameter) filaments in the Müller cells in the same pattern described above for the single-label procedures in both the detached and attached regions. Filaments were present in Müller cell cytoplasm within the ONL of detached retinas and labeled with anti-GFAP and vimentin [Fig. 3(B)]. No label was apparent in Müller cell cytoplasm within the ONL of attached retinas [Fig. 3(A)].

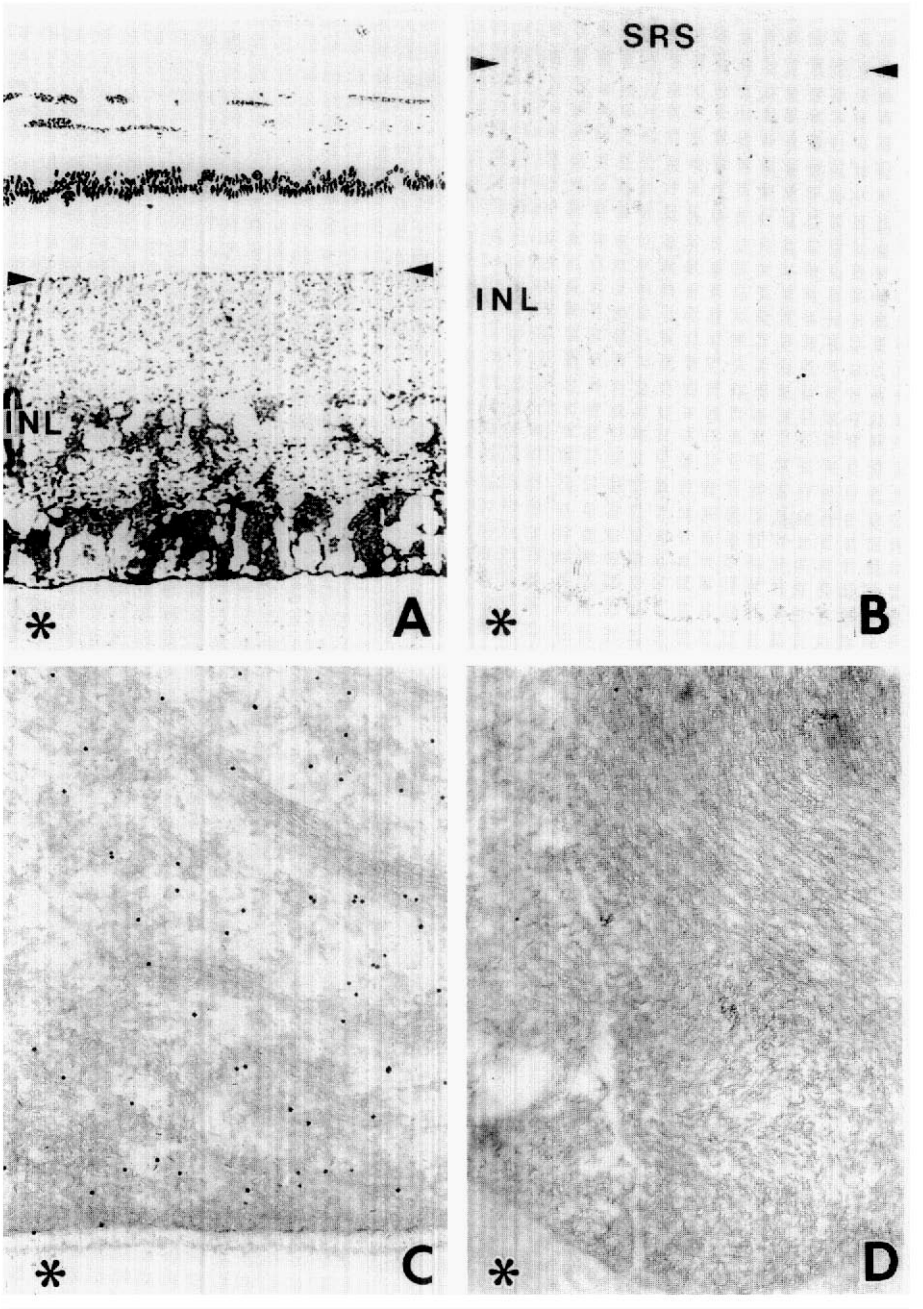

FIG. 4. Light (A, B) and electron (C, D) microscopic immunocytochemistry of anti-GS labeling in 60 day attached (A, C) and detached (B, D) retinas. In attached retinas labeling occurs predominantly in the Müller cells with the heaviest labeling over the endfeet (A, C). (The RPE and choroid in (A) contain pigment granules and no labeling.) After 60 days of detachment there is a dramatic decrease in the label present in the retina (B, D). (* vitreous cavity; INL, inner nuclear layer; arrowheads, OLM; SRS, subretinal space.) Light micrographs ×275; electron micrographs ×4100.

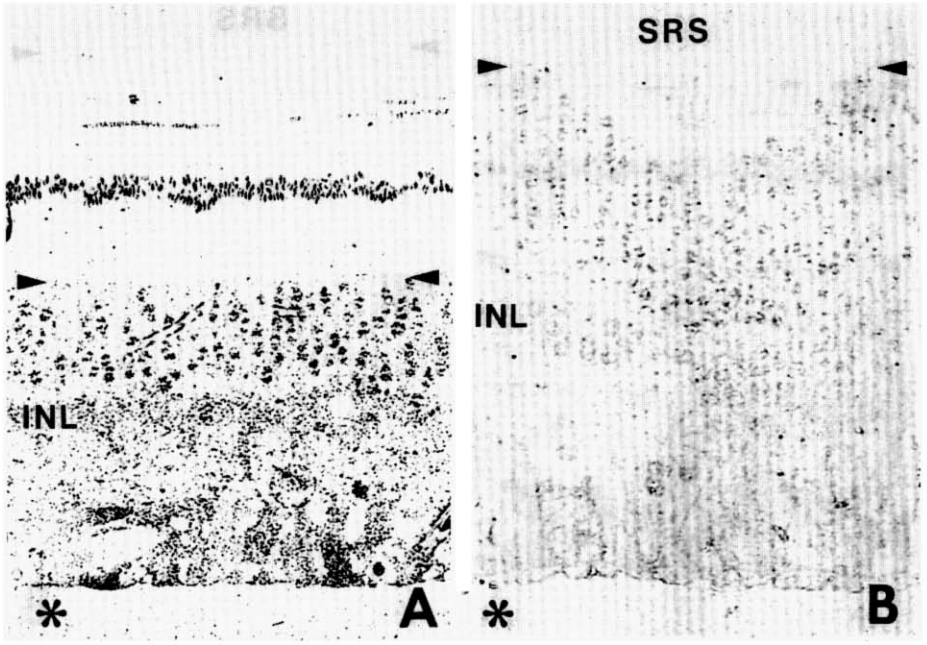

FIG. 5. For legend see opposite.

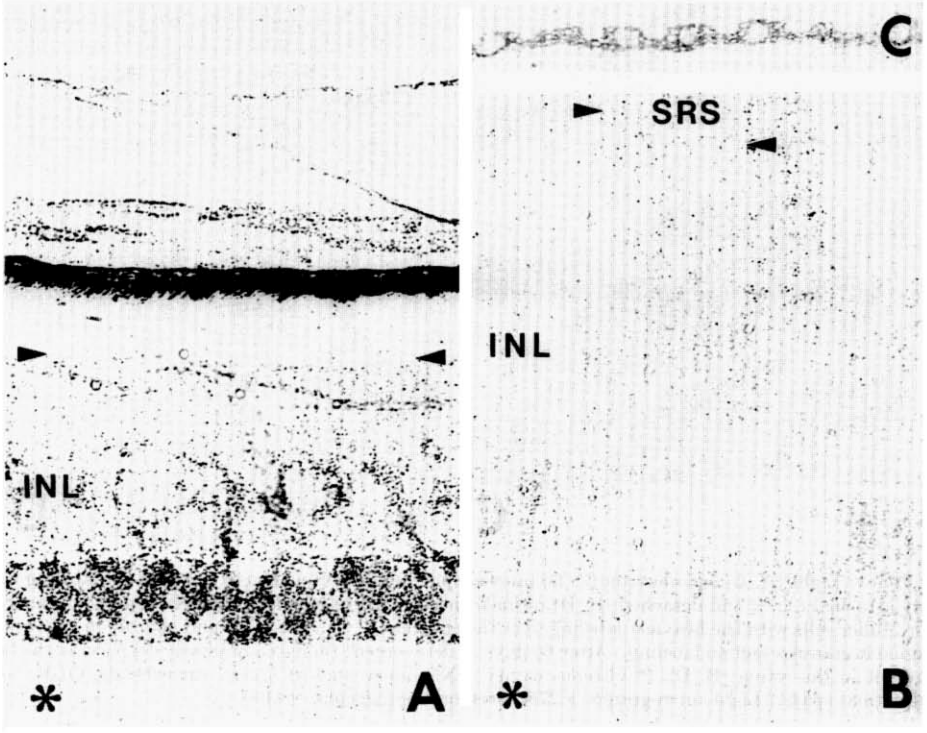

FIG. 6. For legend see opposite.

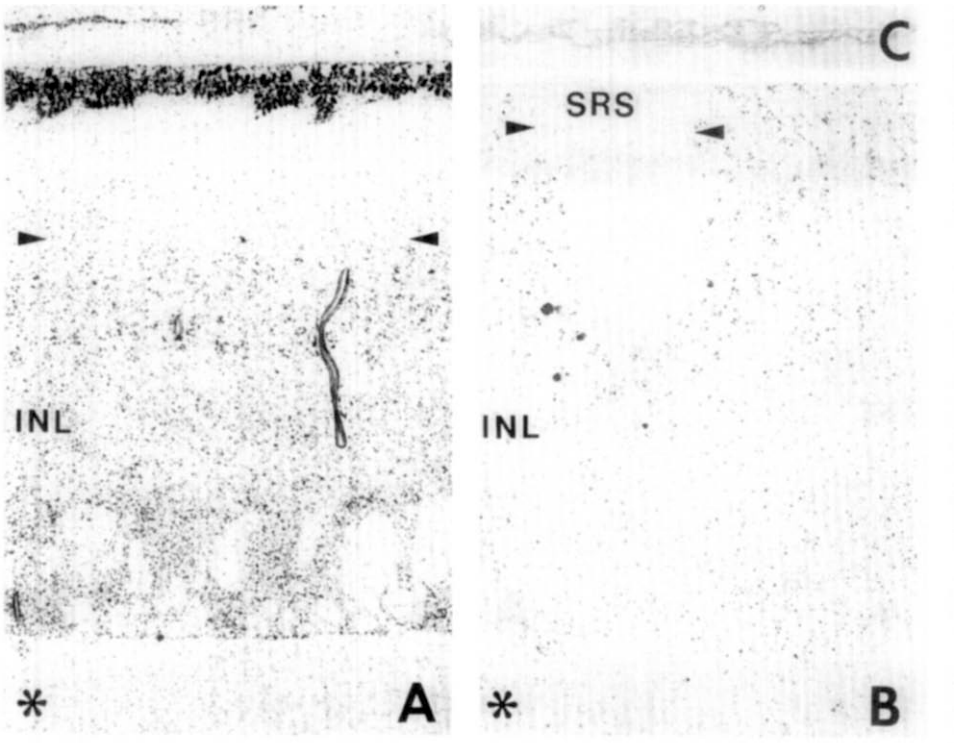

FIG. 7. Light microscopic immunocytochemistry of anti-CRBP labeling in 60 day attached (A) and detached (B) retinas and the RPE in a region of retinal detachment (C). In attached retinas labeling occurs in the Müller cells, the ONL and the RPE. [The RPE in (A) also contains pigment granules.] After detachment there is a reduction in labeling in the retina although there is still some label present in the ONL. The RPE in regions where the retina is detached still labels but not as intensely as in the attached retinas. There are no pigment granules in the RPE in this region of the retina. (* vitreous cavity; INL, inner nuclear layer; arrowheads, OLM; SRS, subretinal space.) ×480.

Immunolabeling with antibodies to GS, CAC, CRALBP and CRBP in attached retinal regions occurred throughout the Müller cell cytoplasm, with the heaviest labeling seen in the endfeet [Figs 4(A), 5(A), 6(A), 7(A)]. Anti-CAC and anti-CRBP labeling was also present in the photoreceptor nuclei and anti-CRALBP and CRBP labeling was found in the RPE cytoplasm. In the detached retinas, however, the labeling with all four antibodies dramatically decreased in the Müller cells [Figs 4(B), 5(B), 6(B), 7(B)]. Some anti-CAC labeling, however, was still apparent

FIG. 5. Light microscopic immunocytochemistry of anti-CAC labeling in 60 day attached (A) and detached (B) retinas. In attached retinas labeling occurs in the Müller cells and photoreceptor nuclei. [The RPE and choroid in (A) contains pigment granules and no labeling.] After 60 days of detachment there is a decrease in CAC labeling in the retina although some label is still apparent in the outer nuclear layer. (* vitreous cavity; INL, inner nuclear layer; arrowheads, OLM; SRS, subretinal space.) ×275.

FIG. 6. Light microscopic immunocytochemistry of anti-CRALBP labeling in 60 day attached (A) and detached (B) retinas and the RPE in a region of retinal detachment (C). In attached retinas labeling occurs in the Müller cells and the RPE. [The RPE in (A) also contains pigment granules as does the unlabeled choroid.] After detachment there is a dramatic decrease in labeling in the retina. The RPE in regions where the retina is detached still labels although not as intensely as in the attached regions [as in (A)]. There are no pigment granules in the RPE in this region of the retina. (* vitreous cavity; INL, inner nuclear layer; arrowheads, OLM; SRS, subretinal space.) ×275.

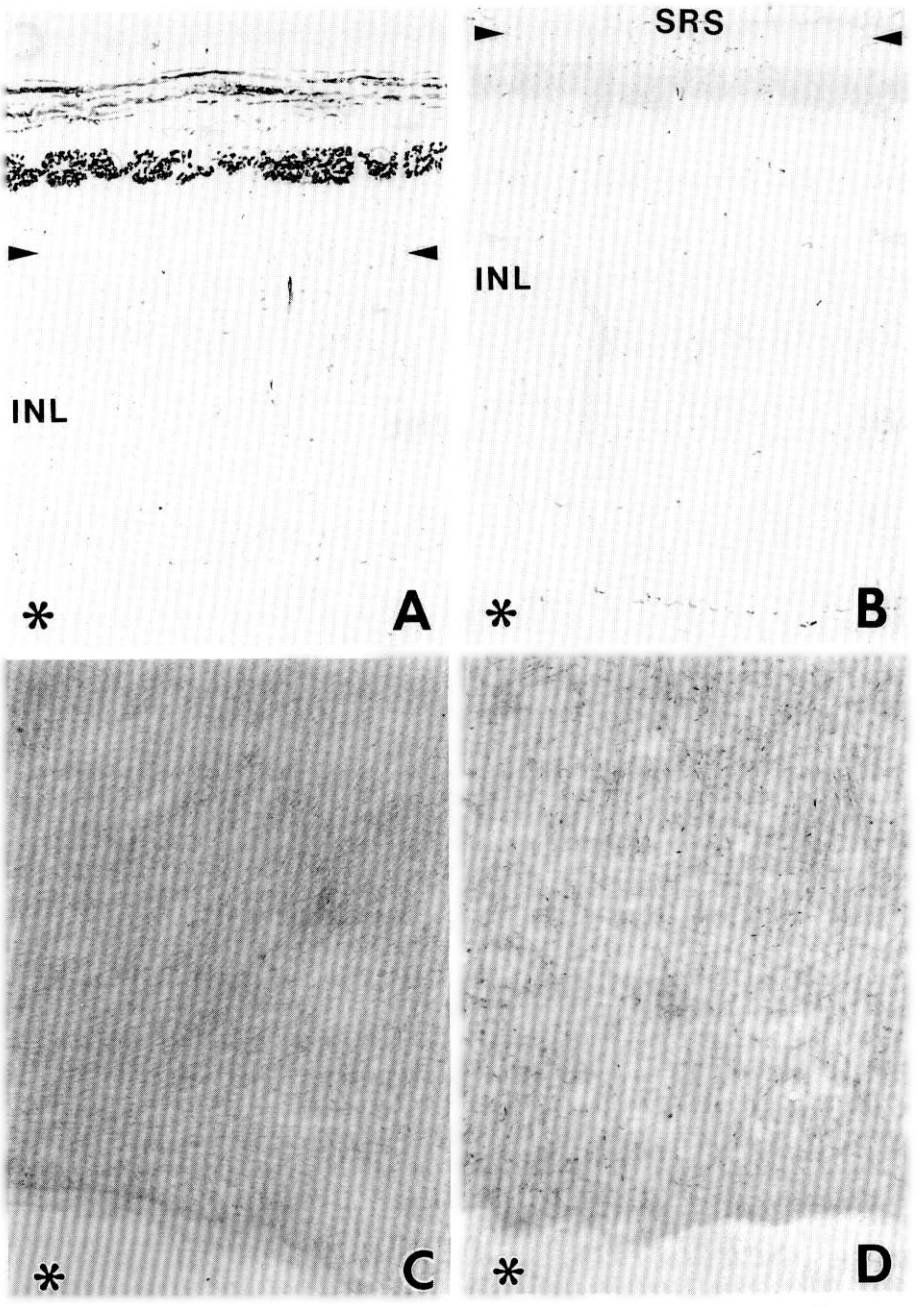

FIG. 8. Control sections of light (A, B) and electron (C, D) microscopic immunocytochemistry from attached (A, C) and detached (B, D) retinas. There are pigment granules present in the RPE and choroid of the attached retina (A) that does not represent labeling. (* vitreous cavity; INL, inner nuclear layer; arrowheads, OLM; SRS, subretinal space.) Light micrographs ×275; electron micrographs ×4100.

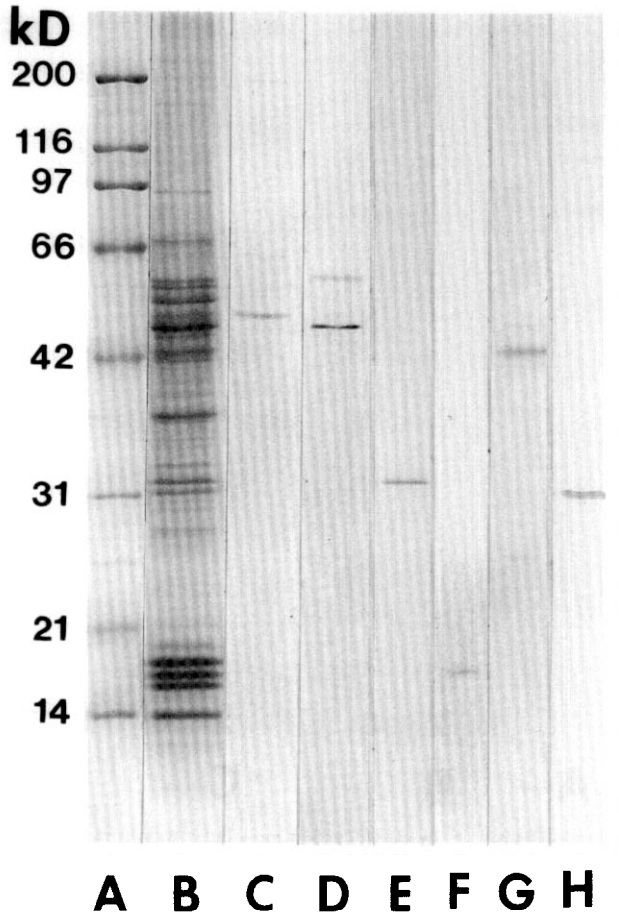

FIG. 9. Coomassie Blue-stained SDS–PAGE gel of molecular weight standards (Lane A) and proteins of cat retinal homogenates (Lane B). Western Blots of normal cat retinal homogenates are in Lanes C–H. Immunoblotting reveals bands at the accepted molecular weights for each of the proteins: anti-GFAP labels a band at 51 kDa (Lane C), anti-vimentin at 57 and 49 kDa (Lane D) (see Discussion regarding the second band), anti-CRALBP at 33 kDa (Lane E), anti-CRBP at 16 kDa (Lane F), anti-GS at 45 kDa (Lane G), and anti-CAC at 31 kDa (Lane H).

in the photoreceptor nuclei; anti-CRALBP and CRBP labeling was still detected in the RPE although it was not as intense as in attached regions [Figs 6(C) and 7(C)]. The same results were obtained for all the antibodies using electron microscopic immunocytochemistry. For the sake of brevity, only the anti-GS labeling is shown [Fig. 4(C)] wherein the endfoot region of the Müller cells showed the heaviest antibody labeling in attached retina, and few if any gold spheres occurred over comparable areas in the region of detachment [Fig. 4(D)]. No label was present on control sections using either the light or electron microscopic techniques (Fig. 8). Similar results were obtained for both the 30- and 60-day time points.

Western blots of homogenates from normal cat retinas showed single bands at the appropriate molecular weights for all the antibodies except that of anti-vimentin: anti-GFAP labeled a band at 51 kDa, anti-CRALBP at 33 kDa, anti-CRBP at 16 kDa, anti-GS at 45 kDa and anti-CAC at 31 kDa (Fig. 9). Anti-vimentin labeled one

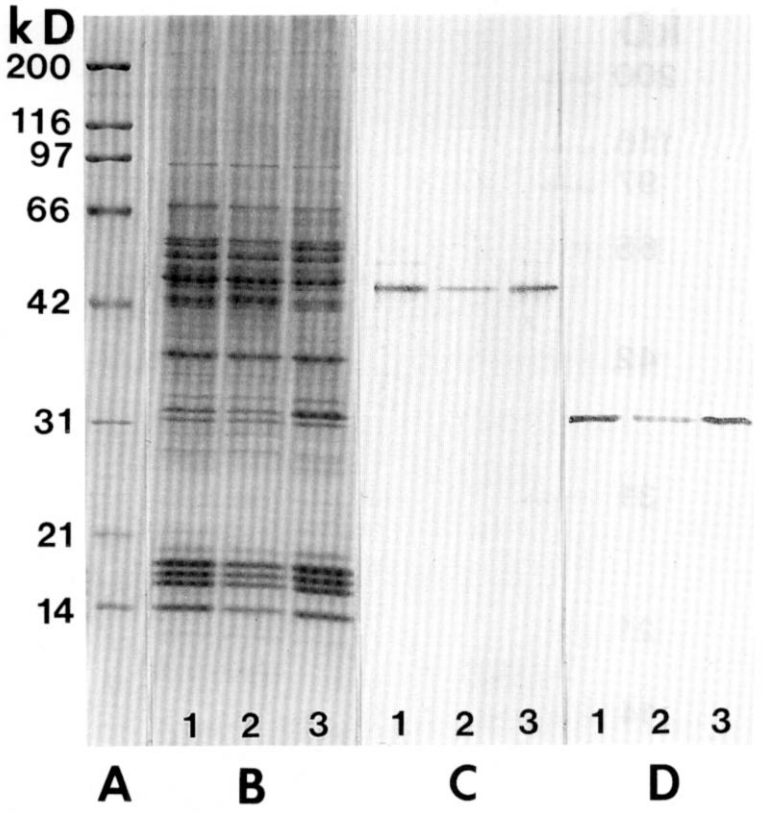

FIG. 10. Coomassie Blue-stained SDS–PAGE gels of molecular weight standards are shown in Panel A and proteins of retinal homogenates from attached, detached and normal retinas are shown in Panel B, Lanes 1–3, respectively. Western Blots of anti-GS (Panel C; attached, detached and normal retinas are shown in Lanes 1–3, respectively) and anti-CAC (Panel D; attached, detached and normal retinas are shown in Lanes 1–3, respectively). Both GS and CAC show a reduction in the intensity of bands using detached retinas (Panels C and D, Lane 2) as compared to the attached and normal retinas.

band at the appropriate molecular weight (57 kDa) and another at approximately 49 kDa. Western blots of attached, detached and normal retinas using antibodies to GS, CAC (Fig. 10), CRALBP and CRBP (Fig. 11) showed a decrease of labeling intensity in the lanes containing samples of the detached retinas, while normal and attached retinas had labeled bands with similar density.

4. Discussion

Retinal Müller cells, a specialized form of astroglia, have been shown to become 'reactive' in a number of ocular diseases. For example, they are suspected of contributing to proliferative vitreoretinopathy by migrating into the vitreous cavity and proliferating (Laqua and Machemer, 1975; Clarkson, Green and Massof, 1977; Machemer, 1988). The cytoplasmic level of GFAP and intermediate filaments has been shown previously to increase in response to penetrating ocular injury (*Bignami* and Dahl, 1979; Miller and Oberdorfer, 1981), light damage (Eisenfeld, Bunt-Milam and Sarthy, 1984), inherited retinal degeneration (Eisenfeld et al., 1984), and retinal

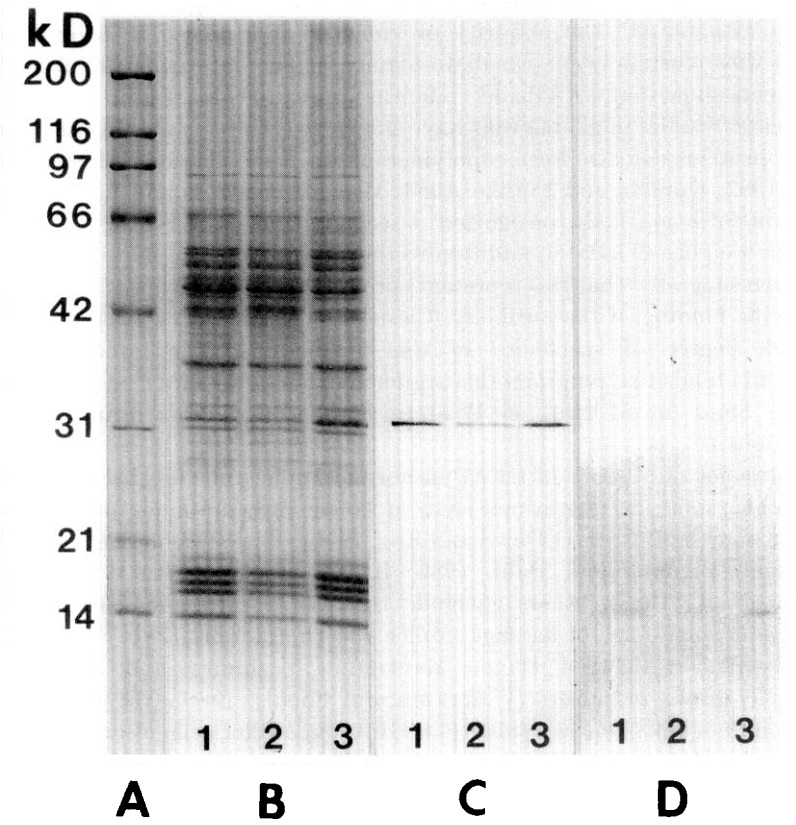

FIG. 11. Coomassie Blue-stained SDS–PAGE gels of molecular weight standards are shown in Panel A and proteins of cat retinal homogenates from attached, detached and normal retinas are shown in Panel B, Lanes 1–3, respectively. Western Blots of anti-CRALBP (Panel C; attached, detached and normal retinas are shown in Lanes 1–3, respectively) and anti-CRBP (Panel D; attached, detached and normal retinas are shown in Lanes 1–3, respectively). Both CRALBP and CRBP show a reduction in the intensity of bands using detached retinas (Panels C and D, Lane 2) as compared to the attached and normal retinas.

detachment (Erickson et al., 1987). In retinal detachment, there is an invasion of the interphotoreceptor space (subretinal space) by blood-borne cells and cells from the neural retina and RPE (Ohkuma, 1972; Inahara, 1973; Machemer and Laqua, 1975; Johnson and Foulds, 1977; Anderson et al., 1981; Anderson et al., 1986a; Erickson et al., 1983). Erickson et al. (1983) used morphological criteria to identify Müller cells as one participant in this response. The results of this study show that a population of GFAP- and vimentin-containing cells appears in the expanded subretinal space during a long-term detachment; labeled processes from these cells often can be traced across the width of the neural retina in a configuration unique to Müller cells. Since Müller cells are the only cells in the cat retina that contain both GFAP and vimentin (Erickson and Fisher, 1988) these results provide conclusive evidence that Müller cells are the retinal cell type whose processes invade the subretinal space after detachment.

Intermediate filaments

Vimentin, an intermediate filament protein (see Steinert and Parry, 1985, for review) has been shown by other investigators to be in the endfoot region of normal

Müller cells in species other than cat (Shaw and Weber, 1984; Schnitzer, 1985). As is the case with GFAP, the majority of vimentin is localized in regions which are invested with clusters of intermediate filaments. The data from the double-labeling experiments show that GFAP and vimentin appear to colocalize to the same clusters of filaments. Studies in glioma cells have shown that these two different intermediate filament proteins can also form copolymers in the same filament (Sharp, Osborn and Weber, 1982; Quinlan and Franke, 1983; Wang, Cairncross and Liem, 1984).

The vimentin antibody recognized a second band on Western blots at a lower molecular weight (49 kDa) in addition to the appropriate size for vimentin (57 kDa). It has been suggested that this represents proteolytic degradation of vimentin and not non-specific binding of the antibody (Ciesielski-Treska, Goetschy and Aunis, 1984). The high degree of specificity of this antibody in the immunocytochemical experiments tends to strengthen this argument as does the fact that we routinely find only one band (at 57 kDa) on Western blots using rabbit retinal homogenates (unpubl. obs.).

The presence or absence of GFAP in normal Müller cells has been a controversial issue. While some investigations show it to be present under normal conditions (Bignami and Dahl, 1979; O'Dowd and Eng, 1979; Ohira, Oshima and Kikuchi, 1984; Björkland, Bignami and Dahl, 1985; Kivela, Tarkkanen and Virtanen, 1986; Erickson et al., 1987), others conclude that GFAP is expressed only in retinas experiencing some form of damage (Miller and Oberdorfer, 1981; Shaw and Weber, 1983; Eisenfeld et al., 1984; Okada, Matsumura, Yoshimura and Ogino, 1988) or in Müller cells grown in culture (Wakakura and Foulds, 1988). We have consistently found immunocytochemical evidence of GFAP in Müller cells of normal cat retinas, both in situ and in culture (Erickson et al., 1987; Lewis, Kaska, Vaughan and Fisher, 1988). The retinas we have classified as normal are from young adult cats (6 months to 2 yr of age) and show no identifiable signs of trauma or degeneration when examined by indirect ophthalmoscopy or light and electron microscopy. The differences reported in the literature may be due to the difficulty in visualizing the relatively small amount of GFAP found in Müller cell endfeet in the normal eye or reflect actual species differences in the expression of these proteins.

The dramatic increase in the expression of intermediate filament proteins represents an important structural response of the Müller cells to retinal injury. Intermediate filaments have been assigned putative functions in the differentiation and maintenance of cell shape, anchoring the nucleus to the cell membrane, and functional compartmentalization of the cytoplasm (Lazarides, 1980, 1987). All of these are relevant to Müller cell behavior after retinal detachment because they change their shape, their nuclei often translocate to new regions and they alter their expression of specific cytoplasmic proteins (Erickson et al., 1983, 1987). The specific function(s) of the intermediate filaments of Müller cells in normal and in pathological retinas, however, remains to be determined.

Müller cell enzymes

Glutamine synthetase and CAC are two enzymes that have been localized to the Müller cells in numerous species, including cat (Lewis et al., 1988), and are thought to play important roles in the physiology of these cells (see Moscona, 1983, and Ripps and Witkowsky, 1985 for reviews). When glutamate, a putative retinal neurotransmitter, is released from neurons it is actively sequestered by Müller cells where GS converts it to glutamine which can freely diffuse across cell membranes and be re-

used by neurons. Norenberg (1982) showed that, after producing lesions in the parietal lobes of rats, GS levels initially increased in astrocytes but eventually decreased to non-detectable levels. Decreased levels of GS activity have also been shown to occur in the retinas of dystrophic mice when compared to their normal littermates (LaVail and Reif-Lehrer, 1971). Although at that time it was not known which cells contained GS, it was concluded that GS was not confined to the photoreceptors and that there was possibly a decay of GS activity in the inner retinal cells. The reduction of GS in the region of detachment may reflect a reduction in synaptic activity and, therefore, enzyme substrate in the detached retina.

Carbonic anhydrase C affects Na^+ and K^+ movement and fluid balance (Carter, 1972). The enzyme converts CO_2 and H_2O to H_2CO_3 which spontaneously dissociates into HCO_3^- and H^+; the proton serves as a regulator of intracellular pH. Disruption of carbonic anhydrase activity by acetozolamide results in altered Müller cell function and a decrease in the b-wave of the ERG in the elasmobranch retina (Hensley, Linser and Cohen, 1987). Decreases in the amount of CAC in the detached retina may have significant effects on the Müller cells' ability to regulate the extracellular environment. Interestingly, Kuwabara and Cogan (1960) showed an increase of oxidative enzymes in Müller cells within retinas that had a detachment. Maintaining normal levels of specific enzymes may therefore be important for proper function and even survival of retinal neurons during detachment and may play a role in the ability of the retina to recover functionally after reattachment. Down-regulation in the amount of these two enzymes in Müller cells may reflect a general decrease in their substrates in the detached retina.

Retinoid binding proteins

The retinoid binding proteins, CRALBP and CRBP have previously been localized by immunocytochemistry to both Müller cells and RPE (Bunt-Milam and Saari, 1983; Saari, Bunt-Milam, Bredberg and Garwin, 1984; Bok, 1985; Eisenfeld, Bunt-Milam and Saari, 1985; Anderson et al., 1986b; Lewis et al., 1988). In addition to a decline in the expression of these proteins in Müller cells after detachment, the levels of CRALBP and CRBP in RPE also appear to be less when compared to adjacent RPE that is still attached to the retina. The correlation between the loss of outer segments and a decline in the levels of two vitamin A binding proteins in Müller cells in experimental retinal detachment is intriguing and this system may prove useful in helping to determine the currently unknown role of these proteins in the Müller cells.

Although antibodies to CRBP and CAC labeled predominantly a diffuse cytoplasmic component of the Müller cells, they also appeared to label the photoreceptor nuclei. CRBP has previously been found in the nucleus of RPE cells where it has been suggested it may be involved in the control of gene expression (Bok, Ong and Chytil, 1984; Chytil and Ong, 1984). Carbonic anhydrase C has also been found in the nuclear fraction of some cells (Karler and Woodbury, 1959). It was shown that nuclear staining may be fixation dependent; when unfixed tissue was used the staining was found only in the cytoplasm (Hansson, 1967, 1968; Lutjen-Drecoll and Lonnerholm, 1981; Lutjen-Drecoll, Lonnerholm and Eichhorn, 1983). These results suggest that nuclear localization of CAC may be due to non-specific diffusion into the nucleus. Because the antibodies labeled a single band on the immunoblot, the nuclear staining most likely does not represent binding to some other molecular species although it is possible that these soluble proteins diffuse into the nucleus after a relatively mild fixation.

5. Conclusions

The immunocytochemical and biochemical analyses are consistent in showing that levels of intermediate filament proteins increase in the detached retina whereas a number of Müller cell enzymes and binding proteins decrease in response to retinal detachment. Significant differences between retina adjacent to the zone of detachment and normal retina in the opposite eye are not apparent. Therefore, these results, and all of our other results to date (Erickson et al., 1985; Erickson, Lewis, Anderson and Fisher, 1988), point toward the conclusion that detachment initiates a localized change in retinal metabolism that is not found in adjacent areas of attached retina.

The changes in protein expression described in these experiments occurred after a relatively lengthy detachment interval (30 to 60 days). Recent data, however, suggest that at least some changes (e.g. in GFAP and RNA synthesis) occur well before that time—as early as 3 days post-detachment (Erickson et al., 1985). We are currently examining the time of onset of these changes, the factors responsible for their initiation, and whether these changes, once started, can be halted or reversed.

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REFERENCES

Aguirre, G., Stramm, L. and O'Brien, P. (1985). Diseases of the photoreceptor cells–pigment epithelial complex: influence of spatial, pigmentation and renewal factors. In *Retinal Degenerations: Experimental and Clinical Studies*. (Eds LaVail, M. M., Hollyfield, J. G. and Anderson, R. E.). Pp. 401–20. Alan R. Liss, Inc.: New York.

Anderson, D. H., Guérin, C. J., Erickson, P. A., Stern, W. H. and Fisher, S. K. (1986a). Morphological recovery in the reattached retina. *Invest. Ophthalmol. Vis. Sci.* **27**, 168–83.

Anderson, D. H., Neitz, J., Kaska, D., Fenwick, J., Jacobs, G. H., and Fisher, S. K. (1986b). Retinoid binding proteins in cone-dominant retinas. *Invest. Ophthalmol. Vis. Sci.* **27**, 1015–26.

Anderson, D. H., Stern, W. H., Fisher, S. K., Erickson, P. A. and Borgula, G. A. (1981). The onset of pigment epithelial proliferation after retinal detachment. *Invest. Ophthalmol. Vis. Sci.* **21**, 10–16.

Bignami, A. and Dahl, D. (1979). The radial glia of Müller in the rat retina and their response to injury. An immunofluorescence study with antibodies to the glial fibrillary acidic (GFA) protein. *Exp. Eye Res.* **28**, 63–9.

Björklund, H., Bignami, A. and Dahl, D. (1985). Immunohistochemical demonstration of glial fibrillary acidic protein in normal rat Müller glia and retinal astrocytes. *Neurosci. Lett.* **54**, 363–68.

Bok, D. (1985). Retinal photoreceptor-pigment epithelium interactions. *Invest. Ophthalmol. Vis. Sci.* **26**, 1659–94.

Bok, D., Ong, D. E. and Chytil, F. (1984). Immunocytochemical localization of cellular retinol binding protein in the rat retina. *Invest. Ophthalmol. Vis. Sci.* **25**, 877–883.

Bunt-Milam, A. H. and Saari, J. C. (1983). Immunocytochemical localization of two retinoid-binding proteins in vertebrate retina. *J. Cell Biol.* **97**, 703–712.

Carter, M. J. (1972). Carbonic anhydrase: isoenzymes, properties, distribution and functional significance. *Biol. Rev.* **47**, 465–505.

Chytil, F. and Ong, D. E. (1984). Cellular retinoid-binding proteins. In *The Retinoids*. (Eds Sporn, M. B., Roberts, A. B. and Goodman, D. S.). Pp. 89–123. Academic Press: Orlando.

Ciesielski-Treska, J., Goetschy, J. and Aunis, D. (1984). Proteolytic degradation of vimentin and glial fibrillary acidic protein in rat astrocytes in primary culture. *Eur. J. Biochem.* **138**, 465–71.

Clarkson, J. G., Green, W. R. and Massof, D. (1977). A histopathologic review of 168 cases of preretinal membrane. *Am. J. Ophthalmol.* **84**, 1–17.

Dowling, J. E. and Sidman, R. L. (1962). Inherited retinal dystrophies in the rat. *J. Cell Biol.* **14**, 73–109.

Eagle, R. C. (1984). Mechanisms of maculopathy. *Ophthalmology* **91**, 613–25.

Eisenfeld, A. J., Bunt-Milam, A. H. and Saari, J. C. (1985). Localization of retinoid-binding proteins in developing rat retina. *Exp. Eye Res.* **41**, 299–304.

Eisenfeld, A. J., Bunt-Milam, A. H. and Sarthy, P. V. (1984). Müller cell expression of glial fibrillary acidic protein after genetic and experimental photoreceptor degeneration in the rat retina. *Invest. Ophthalmol. Vis. Sci.* **25**, 1321–8.

Erickson, P. A., Anderson, D. H. and Fisher, S. K. (1987a). Use of uranyl acetate en bloc to improve tissue preservation and labeling for post-embedding immunoelectron microscopy. *J. Electron Microscopy Tech.* **5**, 303–14.

Erickson, P. A. and Fisher, S. K. (1988). Intermediate filament composition distinguishes cat retinal Müller cells from astrocytes. *Invest. Ophthalmol. Vis. Sci.* **29** (Suppl.), 205.

Erickson, P. A., Fisher, S. K., Anderson, D. H., Stern, W. H. and Borgula, G. A. (1983). Retinal detachment in the cat: the outer nuclear and outer plexiform layers. *Invest. Ophthalmol. Vis. Sci.* **24**, 927–42.

Erickson, P. A., Fisher, S. K., Guérin, C. J., Anderson, D. H. and Kaska, D. D. (1985). Changes in 3-H uridine labeling and GFAP localization in the cat retina after separation of neural retina and retinal pigment epithelium. *Invest. Ophthalmol. Vis. Sci.* **26** (Suppl.), 66.

Erickson, P. A., Fisher, S. K., Guérin, C. J., Anderson, D. H. and Kaska, D. D. (1987b). Glial fibrillary acidic protein in Müller cells after retinal detachment. *Exp. Eye Res.* **44**, 37–48.

Erickson, P. A., Lewis, G. P., Anderson, D. H. and Fisher, S. K. (1988). Cellular proliferation induced by retinal detachment. *J. Cell Biol.* (Abstracts) **107**, 298a.

Hansson, H. P. (1967). Histochemical demonstration of carbonic anhydrase activity. *Histochemie* **11**, 112–28.

Hansson, H. P. (1968). Histochemical demonstration of carbonic anhydrase activity in some epithelia noted for active transport. *Acta Physiol. Scand.* **73**, 427–34.

Hensley, S. H., Linser, P. J. and Cohen, J. L. (1987). The effect of acetazolamide on the ERG b-wave of the skate. *Soc. Neurosci. (abstracts)* **13**, 1049.

Inahara, M. (1973). Studies on the fine structure of experimental retinal detachment. *Acta Soc. Ophthalmol. Jpn.* **77**, 1002.

Johnson, N. E. and Foulds, W. S. (1977). Observations on the retinal pigment epithelium and retinal macrophages in experimental retinal detachment. *Br. J. Ophthalmol.* **61**, 564.

Karler, R. and Woodbury, D. M. (1959). Intracellular distribution of carbonic anhydrase. *Biochem. J.* **75**, 538–43.

Kivela, T., Tarkkanen, A. and Virtanen, I. (1986). Intermediate filament in the human retina and retinoblastoma. An immunohistochemical study of vimentin, glial fibrillary acidic protein and neurofilaments. *Invest. Ophthalmol. Vis. Sci.* **27**, 1075–84.

Kroll, A. J. and Machemer, R. (1968). Experimental retinal detachment in the owl monkey. III. Electron microscopy of retina and pigment epithelium. *Am. J. Ophthalmol.* **66**, 410.

Kuwabara, T. and Cogan, D. G. (1960). Tetrazolium studies on the retina. III. Activity of metabolic intermediates and miscellaneous substrates. *J. Histochem. Cytochem.* **8**, 214–24.

Laqua, H. and Machemer, R. (1975). Glial cell proliferation in retinal detachment (massive periretinal proliferation). *Am. J. Ophthalmol.* **80**, 602–18.

LaVail, M. M. (1981). Analysis of neurological mutants with inherited retinal degeneration. *Invest. Ophthalmol. Vis. Sci.* **21**, 638–68.

LaVail, M. M. and Reif-Lehrer, L. (1971). Glutamine synthetase in the normal and dystrophic mouse retina. *J. Cell Biol.* **51**, 348–54.

Lazarides, E. (1980). Intermediate filaments as mechanical integrators of cellular space. *Nature (London)* **283**, 249–56.

Lazarides, E. (1987). From genes to structural morphogenesis: the genesis and epigenesis of a red blood cell. *Cell* **51**, 345–56.

Lewis, G. P., Erickson, P. A., Kaska, D. D. and Fisher, S. K. (1988). An immunocytochemical comparison of Müller cells and astrocytes in the cat retina. *Exp. Eye Res.* **47**, 839–53.

Lewis, G. P., Kaska, D. D., Vaughan, D. K. and Fisher, S. K. (1988). An immunocytochemical study of cat retinal Müller cells in culture. *Exp. Eye Res.* **47**, 855–68.

Lutjen-Drecoll, E. and Lonnerholm, G. (1981). Carbonic anhydrase distribution in the rabbit eye by light and electron microscopy. *Invest. Ophthalmol. Vis. Sci.* **21**, 782–97.

Lutjen-Drecoll, E., Lonnerholm, G. and Eichhorn, M. (1983). Carbonic anhydrase distribution in the human and monkey eye by light and electron microscopy. *Albrecht von Graefes Arch. Klin. Exp. Ophthalmol.* **220**, 285–91.

Machemer, R. (1988). Proliferative vitreoretinopathy (PVR): a personal account of its pathogenesis and treatment. *Invest. Ophthalmol. Vis. Sci.* **29**, 1771–83.

Machemer, R. and Laqua, H. (1975). Pigment epithelial proliferation in retinal detachment (massive periretinal proliferation). *Am. J. Ophthalmol.* **80**, 1.

Miller, N. M. and Oberdorfer, M. (1981). Neuronal and neuroglial responses following retinal lesions in the neonatal rats. *J. Comp. Neurol.* **202**, 493–504.

Moscona, A. A. (1983). On glutamine synthetase, carbonic anhydrase and Müller glia in the retina. In *Progress in Retinal Research*, Vol 2. (Eds Osborne, N. and Chader, G.). Pp. 111–35. Pergamon Press: Oxford.

Norenberg, M. D. (1982). Immunohistochemical study of glutamine synthetase in brain trauma. *J. Neuropathol. Exp. Neurol.* **41**, 347.

Nork, T. M., Wallow, I. H. L., Sramek, S. J. and Anderson, G. L. (1987). Müller cell interaction in proliferative diabetic retinopathy. *Invest. Ophthalmol. Vis. Sci.* **28** (Suppl.), 123.

O'Dowd, D. K. and Eng, L. F. (1979). Immunocytochemical localization of the glial fibrillary acidic (GFA) protein in the Müller cells of the human retina. *Soc. Neurosci. Abstr.* **5**, 431.

Ohira, A., Oshima, K. and Kikuchi, M. (1984). Localization of glial fibrillary acidic (GFA) protein in the human Müller cell. *Nippon Ganka Gakkai Zasshi* **88**, 1068–74.

Ohkuma, M. (1972). Transmission and scanning electron microscopic observations of the experimentally detached retina in the rabbit. I. Early changes of the detached retina. *Acta Soc. Ophthalmol. Jpn.* **76**, 303.

Okada, M., Matsumura, M., Yoshimura, N. and Ogino, N. (1988). Human Müller cells express glial fibrillary acidic protein in proliferative vitreoretinopathy. *Invest. Ophthalmol. Vis. Sci.* **29** (Suppl.), 305.

Quinlan, R. A. and Franke, W. W. (1983). Molecular interactions in intermediate-sized filaments revealed by chemical cross-linking. *Eur. J. Biochem.* **132**, 477–84.

Rayborn, M. E., Frederick, J. M., Ulshafer, R. S., Tabor, G. A., Moorhead, L. C. and Hollyfield, J. G. (1985). Histopathology and in vitro metabolic studies on tissues from a family with an autosomal dominant form of retinitis pigmentosa. In *Retinal Degenerations: Experimental and Clinical Studies*. (Eds LaVail, M. M., Hollyfield, J. G. and Anderson, R. E.). Pp. 37–49. Alan R. Liss, Inc: New York.

Ripps, H. and Witkowsky, P. (1985). Neuron–glia interaction in the brain and retina. In *Progress in Retinal Research*, Vol. 4. (Eds Osborne, N. and Chader, G.). Pp. 181–219. Pergamon Press: Oxford.

Saari, J. C., Bunt-Milam, D., Bredberg, L. and Garwin, G. G. (1984). Properties and immunocytochemical localization of three retinoid-binding proteins from bovine retina. *Vis. Res.* **24**, 1595–1603.

Sarthy, P. V. and Fu, M. (1988). Induction of glial fibrillary acidic protein (GFAP) gene in the mouse retina. *Invest. Ophthalmol. Vis. Sci.* **29** (Suppl.), 169.

Schnitzer, J. (1985). Distribution and immunoreactivity of glia in the retina of the rabbit. *J. Comp. Neurol.* **240**, 128–42.

Sharp, G., Osborn, M. and Weber, K. (1982). Occurrence of two different intermediate filament proteins in the same filament in situ within a human glioma cell line. *Exp. Cell Res.* **141**, 385–95.

Shaw, G. and Weber, K. (1983). The structure and development of the rat retina: an immunofluorescence microscopical study using antibodies specific for intermediate filament proteins. *Eur. J. Cell Biol.* **30**, 219–32.

Shaw, G. and Weber, K. (1984). The intermediate filament complement of the retina: a comparison between different mammalian species. *Eur. J. Cell Biol.* **33**, 95–104.

Steinert, P. M. and Parry, D. A. D. (1985). Intermediate filaments: conformity and diversity of expression and structure. *Ann. Rev. Cell Biol.* **1**, 41–65.

Wakakura, M. and Foulds, W. S. (1988). Immunocytochemical characteristics of Müller cells cultured from adult rabbit retina. *Invest. Ophthalmol. Vis. Sci.* **29**, 892–900.

Wang, E., Cairncross, J. G. and Liem, R. K. H. (1984). Identification of glial filament protein and vimentin in the same intermediate filament system in human glioma cells. *Proc. Natl. Acad. Sci. U.S.A.* **81**, 2102–6.