Fucosylated Protein of Retinal Cone Photoreceptor Outer Segments: Morphological and Biochemical Analyses

ANN H. BUNT and JOHN C. SAARI
Department of Ophthalmology, University of Washington, Seattle, Washington 98195

ABSTRACT Cone outer segments (OS) of the goldfish retina are diffusely labeled after intravitreal injection of [^{3}H]fucose while rod OS remain unlabeled. By electron microscopic radioautography, the OS of red- and blue-sensitive cones are heavily labeled while green-sensitive cone OS are lightly labeled. The time-course and pattern of OS labeling in all cone types from 30 min to 24 h resemble that of incorporation of other sugars into rhodopsin in rod OS. The nature of the cone OS-specific fucosylated component(s) was examined using biochemical techniques. Cone OS were prelabeled by intravitreal injection of [^{3}H]fucose 24 h before sacrifice. Photoreceptor OS were isolated using a discontinuous sucrose density gradient and it was verified by electron microscopic radioautography that the only source of radioactivity in the preparations was cone OS. The different cone types could be recognized by the heaviness of labeling, characteristic membrane spacing, and "staining" of green cone OS in vitro with horseradish peroxidase. After acid hydrolysis of prelabeled photoreceptor membranes, 90% of the counts were in the neutral sugar fraction which was analyzed by thin-layer chromatography. ~70% of the radioactivity co-chromatographed with authentic fucose. SDS-PAGE/fluorography of prelabeled photoreceptor membranes revealed a single radioactive component that was lightly stained with Coomassie Blue and showed an apparent molecular weight of 33,000. This cone-derived band was separated from unlabeled rod opsin which was well stained and showed an apparent mol wt of 38,000. Isoelectric focusing under denaturing conditions produced two major and one minor band of radioactivity with isoelectric points of 8.2, 8.6, and 8.8, respectively. No radioactivity was found in association with a stained band corresponding in isoelectric point to that of bovine opsin (pI, 6.2). The fucosylated component was readily digested by pronase, indicating its protein nature. Washing of the isolated OS with isotonic and hypotonic buffers failed to extract major amounts of the radioactivity, suggesting that the fucosylated component is an integral membrane protein. The presence of a fucosylated protein thus represents a major difference between cone and rod OS in the goldfish and has enabled us to identify cone OS in preparations of isolated photoreceptor membranes and to demonstrate the separation of a cone-derived glycoprotein from rod opsin.

Recent studies have indicated that the surface membrane saccharide composition may vary among closely associated cells of different types within a given tissue (1, 2), including the retina (3, 4, 5). Although rods and cones of the vertebrate retina exhibit major differences in morphology and spectral sensitivities, relatively little is known about the biochemical properties of the surface membranes of these two major classes of photoreceptors (3, 6). In these cells, the plasma membrane is specialized to form the OS which in the rod contains numerous membranous discs formed by pinching off from the surface membrane. Cone OS are composed of multiple infoldings of the plasma membrane which remain open to the extracellular space. The major structural protein of the rod OS membrane is opsin, a glycoprotein which in bovine rods contains mannose and *N*-acetylglucosamine but not fucose (7, 8, 9, 10). Comparable information on the saccharide content of cone visual pigments is not available; however, chicken iodopsin has been shown to bind to concanavalin-A-sepharose and is most likely a glycoprotein (11).

Fucosylated glycoproteins are common constituents of plasma membranes of a variety of cell types. Recently (12, 13), it was shown that cone but not rod OS in a number of species

THE JOURNAL OF CELL BIOLOGY · VOLUME 92 FEBRUARY 1982 269–276
© The Rockefeller University Press · 0021-9525/82/02/0269/08 $1.00

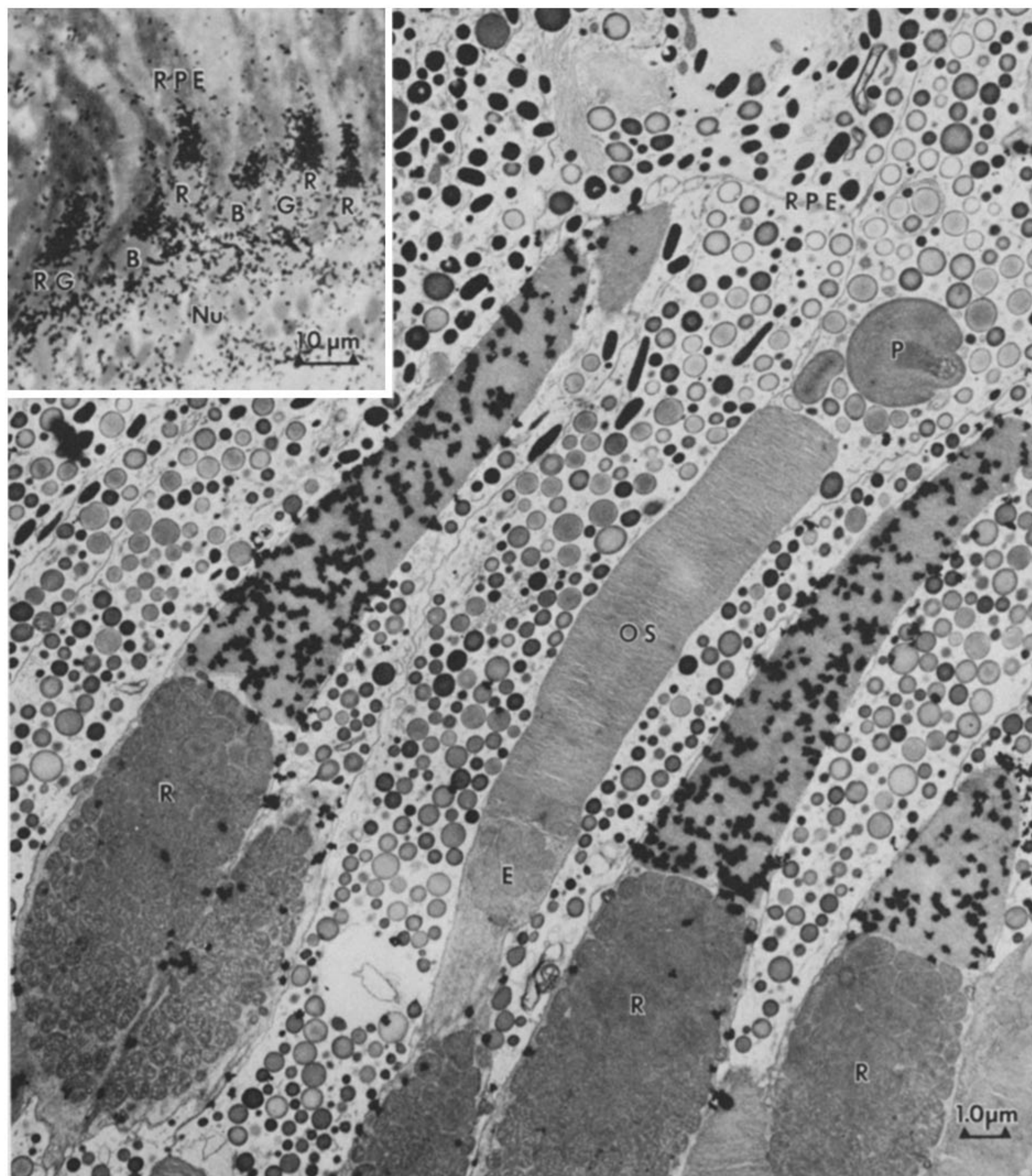

FIGURE 1 Inset: Light microscopic radioautograph of Black Moor goldfish cones 24 h after intravitreal injection of [^{3}H]fucose. Note heavy, diffuse labeling of outer segments of red- (*R*) and blue- (*B*) sensitive cones and light labeling of green- (*G*) sensitive cone outer segments. *RPE*, retinal pigment epithelium; *Nu*, outer nuclear layer. (× 1,500). Electron microscopic radioautograph shows heavily labeled outer segments of red-sensitive cones (*R*) and an unlabeled rod outer segment (*OS*). *E*, rod ellipsoid; *P*, rod derived phagosome in retinal pigment epithelium (*RPE*). × 7,500.

become diffusely labeled after intravitreal injection of [^{3}H]-fucose. In goldfish, a species of interest because of the large size of the cone photoreceptors (14), the OS of red- and blue-sensitive cones were heavily labeled with [^{3}H]fucose while green-sensitive cone OS were lightly labeled, as documented by light and electron microscopic radioautography. In the present study we have examined the time-course and pattern of labeling of the cone OS and the biochemical characteristics of the fucosylated components that are specific to cone OS. The presence of fucose in cone but not rod OS has enabled the identification of cone OS in isolated photoreceptor membrane preparations and allowed us to demonstrate separation of a cone-derived glycoprotein from rod opsin.

MATERIALS AND METHODS

Morphologic Studies

Comet and Black Moor goldfish (*Carassius auratus*) were anesthetized with 0.5% (wt/vol) tricaine methane sulfonate (Ayerst Laboratories, Inc., New York, N. Y.) in tank water. The eyes were injected intravitreally with 10 μl of L-[5,6-^{3}H]-fucose (60 Ci/mmol; New England Nuclear, Boston, Mass., 10 μCi/ul sterile isotonic saline). After survival periods of 30, 60, or 90 min, 3, 4, or 24 h, the fish were decapitated, pithed, and the eyes were fixed by immersion in 1% glutaraldehyde and paraformaldehyde or with 1% paraformaldehyde alone, in

0.1 M phosphate buffer at pH 7.4 for 24 h at 4°C. Samples of retina were dissected out, rinsed in buffer, postfixed with OsO_4 in phosphate buffer, dehydrated through an ethanol series, and embedded in Epon. Sections were processed for light and electron microscopic radioautography as described previously (12).

Preparation of Photoreceptor Membranes

Black Moor goldfish were injected intravitreally with [^{3}H]fucose as described above. After 24 h, dark-adapted fish were decapitated under dim red illumination, pithed, and the retinae were dissected free of vitreous humor and collected in chilled (0°C) 34% sucrose (wt/vol), 0.01 M Tris-HCl, pH 7.4, 1 mM $MgCl_2$, 1 mM dithiothreitol, and 10 μg/ml α-tocopherol (0.5 ml per retina). The procedure of Papermaster and Dreyer (15) was followed for the preparation of photoreceptor OS, except that the retinae were homogenized at 5°C under dim red illumination by four passes each through 13 and 18 gauge trochars. Dithiothreitol (1 mM) was included in all buffers and 10 μg/ml α-tocopherol was included at each stage of membrane resuspension. After centrifugation in a discontinuous sucrose density gradient (15), samples of photoreceptor membranes were collected from the 1.11/1.13 and 1.13/1.15 g/ml interfaces and monitored by light and electron microscopic radioautography as described previously (16). For in vitro "staining", photoreceptor membranes were incubated at 5°C with 5 mg/ml horseradish peroxidase (type VI, Sigma Chemical Co., St. Louis, Mo.) in 0.15 M KCl, 0.1 M phosphate buffer, pH 7.5, for 15 min, reisolated by sucrose density gradient centrifugation, fixed as described above for 1 h, reacted cytochemically for the demonstration of peroxidase activity (17), and processed for electron microscopic radioautography as above.

Hydrolysis and Identification of Sugars

Samples of [^{3}H]fucose-labeled photoreceptor membranes were hydrolyzed in 1 N HCl at 100°C for 16 h in sealed, evacuated tubes that had been flushed with N_2. Carrier mannose and fucose were added to the hydrolysate which was neutralized with Dowex 1-X8(HCO_3^-) and passed through a column of Dowex 50-X2 (H^+ form) to remove charged substances. The eluate was dried, taken up in a small volume of water, and applied to a 5 × 35 cm thin-layer plate of silica gel G prepared in 0.3 M phosphate buffer, pH 8. After developing the plate in *n*-butanol: acetone:H_2O (4:5:1.6, vol/vol) for ~4 h, sugars were revealed by heating the plate at 90°C for 5 min after spraying with triphenyl tetrazolium chloride in ethanol (18). Tritium was localized by liquid scintillation counting of silica gel scraped from the plate.

Proteolysis of OS

OS's prelabeled with [^{3}H]fucose as described above were incubated at pH 7.4 and room temperature for 15 min after addition of 30 μg/ml pronase (Sigma Chemical Co.). Samples were then chilled to 0°C, centrifuged, and washed before analysis by SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

Gel Electrophoresis

SDS-PAGE was carried out on 15 × 19 × 0.2 cm slabs of gel using the buffer system, sample application buffer, and staining protocol of Fairbanks, Steck, and Wallach (19). Tritium was located in these and the isoelectric focusing gels described below either by liquid scintillation counting of 2-mm slices of gel that had been dissolved by heating in 0.5 ml of 30% H_2O_2 (16) or by fluorography (20) as described below. Isoelectric focusing in slabs of polyacrylamide gels was carried out using the solutions and procedure described by O'Farrell (21), with 2% Triton X-100 substituted for nonidet P-40 (NP-40). Gels were stained with 0.04% (wt/vol) Coomassie Blue R-250, 0.05% (wt/vol) Crocein Scarlet and 0.5% (wt/vol) $CuSO_4$ in 27% (vol/vol) isopropanol and 10% (vol/vol) acetic acid (22). After staining with Coomassie Blue, some gels were impregnated with Enhance (New England Nuclear), dried under vacuum onto Whatman 3-mm filter paper (Whatman, Inc., Clifton, N. J.), and placed in contact with x-ray film which was processed photographically after an exposure of 1 d to 3 wk in a light-tight cassette at −80°C. Isoelectric focusing was performed under normal lighting conditions because the presence of 9 M urea in the dissolution and gel solutions resulted in bleaching of OS preparations.

Washing of OS

Isolated OS were washed three times by resuspension and centrifugation with nearly isotonic buffer (10 mM Tris-HCl, pH 7.5, 0.1 M NaCl, 5 mM $MgCl_2$, 1 mM dithiothreitol) and three times with hypotonic buffer (10 mM Tris-HCl, 5 mM $MgCl_2$, 1 mM dithiothreitol). The amount of radioactivity in each wash relative to the initial amount was determined by liquid scintillation counting.

RESULTS

Cone OS Labeling In Vivo

As reported previously (12, 13), cone OS are labeled diffusely but rod OS remain unlabeled 24 h after intravitreal injection

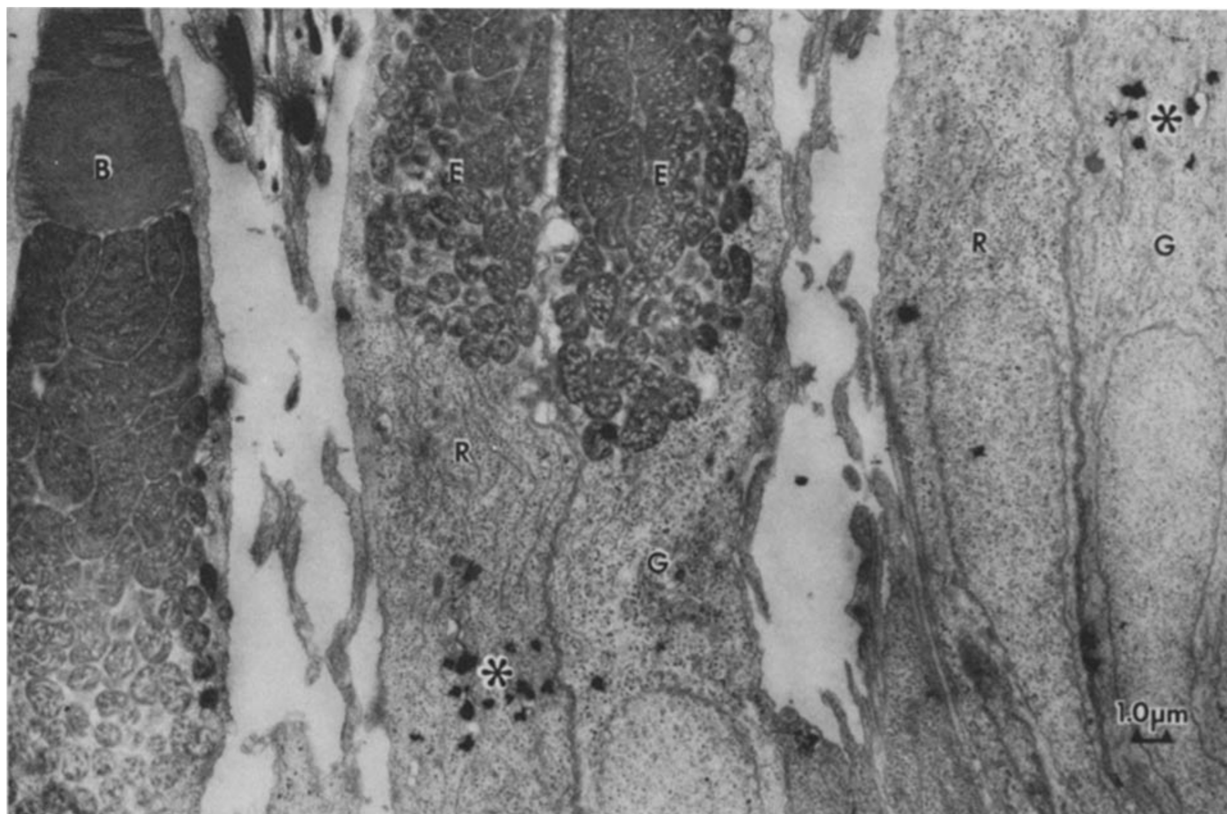

FIGURE 2 Electron microscopic radioautograph of red- (*R*) and green- (*G*) sensitive cones 30-min after intravitreal injection of [^{3}H]fucose. Note initial localization in the Golgi (*) region beneath the ellipsoid (*E*). *B*, blue-sensitive cone. × 5,800.

of [^{3}H]fucose (Fig. 1). Among the different cone types that are identifiable morphologically (23), the OS are heavily labeled in the red- and blue-sensitive cones and lightly labeled in the green-sensitive cones (Fig. 1, inset). In goldfish sacrificed at 30 min postinjection, initial incorporation of [^{3}H]fucose into macromolecules occurred in the Golgi complex (Fig. 2), followed by movement through the ellipsoid and appearance in the OS by 1.5 h. The labeling of the OS and synapse region of all cone types continued to increase over the next 24 h. Label was also prominent over the so-called "accessory OS" which is a membrane-bounded extension off the connecting cilium found here in all cone types but not in rods (Fig. 3). In addition to differential OS labeling, [^{3}H]fucose incorporation into the cone pedicles was much higher than into rod spherules, although postsynaptic elements of both photoreceptor types showed variable labeling.

Photoreceptor Membrane Preparations

Photoreceptor membranes that had been prelabeled with [^{3}H]fucose were collected from the 1.11/1.13 and 1.13/1.15 g/ml interfaces of a discontinuous sucrose density gradient. It was verified by electron microscopic radioautography that the only source of radioactivity in the preparations was cone OS (Fig. 4). Red- and blue-sensitive cone OS, which were recognizable on the basis of membrane spacing (12), were always heavily labeled (Fig. 4). Green-sensitive cone OS were occasionally recognizable on the basis of membrane spacing (12) but usually appeared quite vesiculated, along with the characteristic light pattern of labeling (Fig. 5). The identity of the vesiculated structures as green-sensitive cone OS was also supported by tbe observation that they were "stained" selectively in vitro with horseradish peroxidase (Fig. 6), an extension of the previously observed selective labeling of green-sensitive cone OS with this enzyme in vivo (12). Rod OS were identifiable by the presence of incisures and a light punctate osmium precipitate on the disc membranes; they were always unlabeled with [^{3}H]fucose (Figs. 4–6). The OS from the 1.11/1.13 g/ml interface of the discontinuous sucrose gradient were less intact and osmiophilic than the OS from the 1.13/1.15 g/ml interface, which were consistently more electron-dense and were enriched ~ fourfold in radioactivity and cone OS relative to total outer segments, as quantified from 1-μm sections by light microscopic radioautography. The inclusion of the antioxidants α-tocopherol and dithiothreitol during the isolation procedure improved the yield of OS with preserved morphologic integrity in both fractions.

Identification of Fucose in Labeled Photoreceptor Membranes

To ensure that the [^{3}H]fucose was not being metabolized to other substances, labeled OS were acid hydrolyzed and the neutral sugar fraction was isolated. 90% of the total radioactivity remained in the neutral sugar fraction which was analyzed by thin-layer chromatography. ~70% of the radioactivity cochromatographed with authentic fucose (Fig. 7). The remainder of the counts did not coincide with mannose but may correspond to a disaccharide which contains fucose.

SDS-PAGE

Fig. 8 illustrates a typical example obtained with solubilized goldfish photoreceptor membranes from the 1.11/1.13 and 1.13/1.15 g/ml interfaces. Nearly all of the radioactivity was localized to a single component in the fluorogram that had an apparent mol wt of 33,000. This region stained only lightly with Coomassie Blue in contrast to the heavily stained opsin band (apparent mol wt of 38,000) which contained no radioactivity. Bovine opsin in this system showed an apparent mol wt of 36,000. Fig. 8 also illustrates that the photoreceptor membrane fraction derived from the 1.13/1.15 g/ml interface contains more of the fucosylated component relative to opsin than does the fraction originating from the 1.11/1.13 g/ml interface, a finding in accord with the morphologic observation that the former fraction was enriched ~ fourfold in cone OS. Gels were also sliced and counted before and after staining and destaining procedures. No difference was noted in the pattern obtained, indicating that no radioactive low-mol/wt components, including phospholipids, were lost during these procedures. No radioactivity was associated with the region of the gel immediately behind the tracking dye, known to contain the bulk of the membrane lipid.

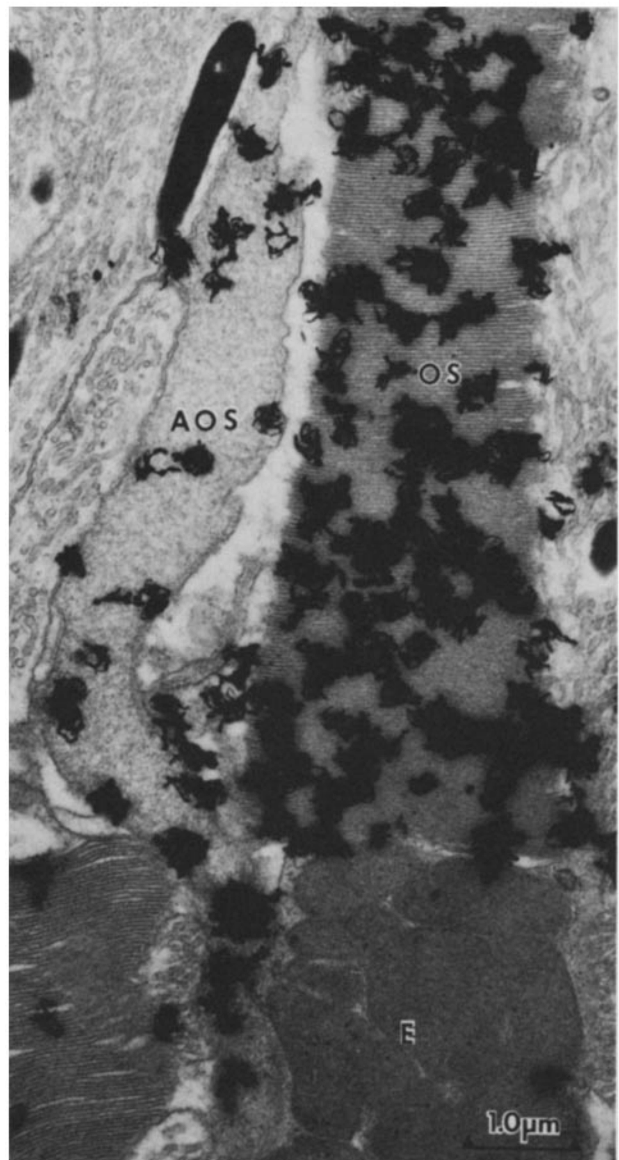

FIGURE 3 At 24-h after intravitreal injection of [^{3}H]fucose, the outer segment (*OS*) and "accessory outer segment" (*AOS*) are labeled in this red cone and other cone types. *E*, ellipsoid. × 16,400.

Incubation of isolated [^{3}H]fucose-labeled photoreceptor membranes with pronase resulted in the disappearance of the labeled band at an apparent mol wt of 33,000 and the appearance of several additional bands of lower mol wt (~15–17,000),

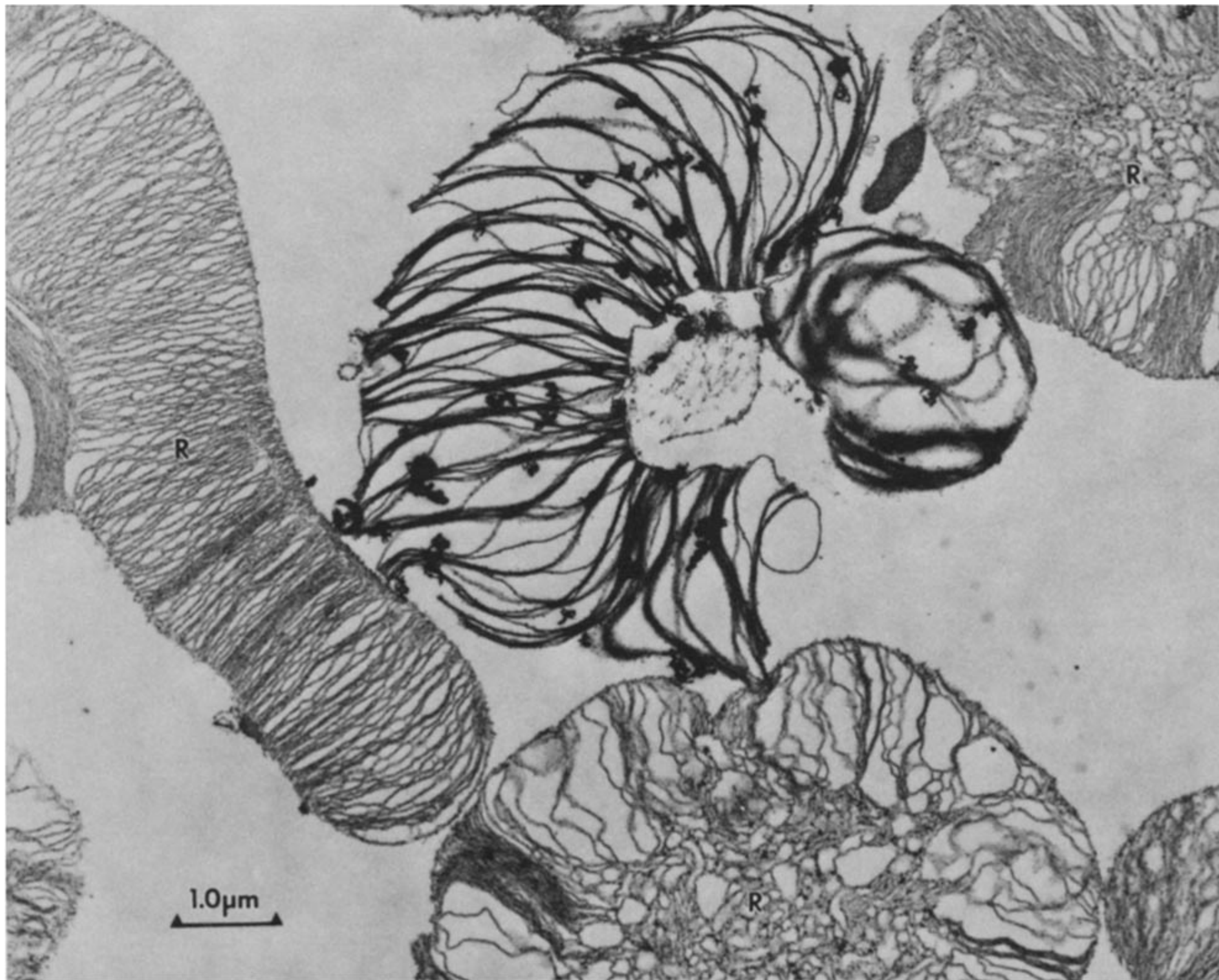

FIGURE 4 Electron microscopic radioautograph of isolated photoreceptor membranes from goldfish retinae prelabeled in vivo with [^{3}H]fucose. Note well labeled red-sensitive cone outer segment and absence of label over rod (*R*) outer segments which show light osmium precipitates on disc membranes. × 15,500.

consistent with the identification of the cone-derived fucosylated material as a glycoprotein.

Isoelectric Focusing of Solubilized Photoreceptor Membranes

Fluorograms of Coomassie Blue-stained isoelectric focusing gels, run in the presence of 8 M urea and Triton X-100, revealed two major radioactive protein bands at pH 8.2 and 8.6 and a minor band at pH 8.8 (Fig. 9). The third, minor band was not present in the analysis of all samples. A stained band at an isoelectric point (pI) of 6.2 was unlabeled and was separated from the fucosylated protein bands. Bovine opsin has been reported to have a pI close to this value (24). The radioactive components apparent in the fluorograms (Fig. 9, *lanes 2* and *4*) correspond to bands on the gel which stain well with Coomassie Blue (Fig. 9, *lanes 1* and *3*). This is in contrast to the result obtained by SDS-PAGE (Fig. 8) where the radioactive component did not correspond to a heavily stained band. The explanation for this is not apparent, although it should be pointed out that the staining procedures differ for the two systems.

Solubility Characteristics of the Fucosylated Component(s)

Goldfish photoreceptor membranes that had been prelabeled in vivo with [^{3}H]fucose were subjected to a washing procedure to determine the solubility of the fucosylated components. Only a small amount of radioactivity (2–4% per wash) was removed during three nearly iso- and three hypotonic washes known to remove soluble components of bovine rod OS (25, 26). In addition, the fluorograms of SDS gels of the washed photoreceptor membrane preparations were not altered as a result of the extractions.

DISCUSSION

The specificity of [^{3}H]fucose labeling of different classes of goldfish cone OS has enabled their recognition in preparations of isolated photoreceptor membranes and separation of a cone-specific glycoprotein from rod opsin. The morphologic appearance of the different cone OS types in vitro agreed well with previous descriptions of their lamellar membrane spacing and "staining" with horseradish peroxidase in vivo. Although the morphologic integrity of the red- and blue-cone OS was relatively well preserved in vitro, the characteristic vesiculation of the green-sensitive cone OS may reflect a difference in adhesion of the lamellar membranes which renders them more sensitive to disruption by the isolation procedure. The possible basis for the OS "staining" with horseradish peroxidase in vitro as well as in vivo (27) will be the topic of a future report.

[^{3}H]fucose is incorporated into the cone OS without modi-

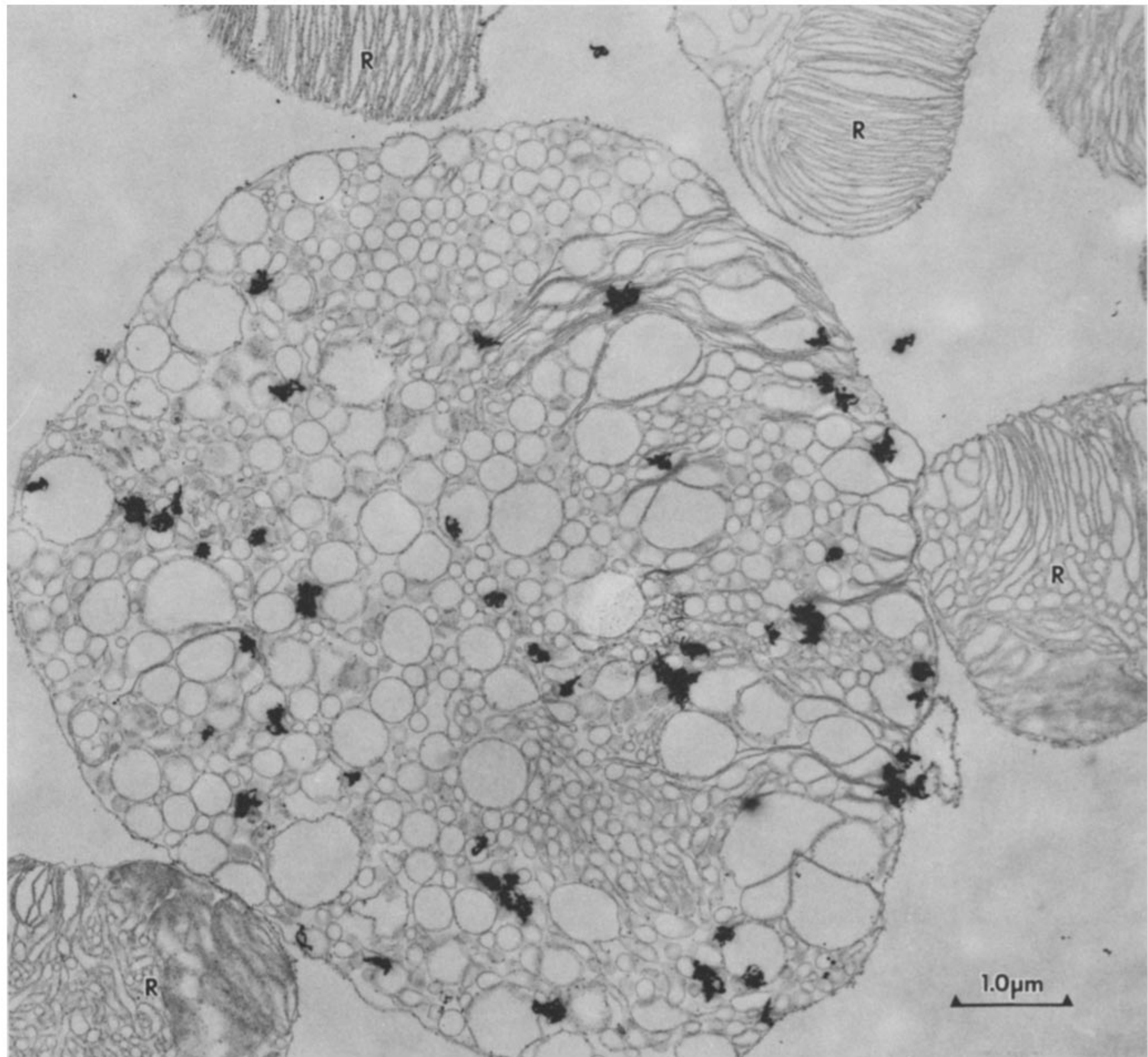

FIGURE 5 The green-sensitive cone outer segments, one of which is illustrated here, usually appeared vesiculated and are lightly labeled with [^{3}H]fucose. *R*, unlabeled rod outer segments. × 18,900.

fication to other precursors, as shown previously for [^{3}H]fucose in rabbit photoreceptor OS (16) and in other tissues (28). The pattern of incorporation of the label into cone OS is also consistent with that shown for other cell types, that is, initial fucosylation of protein occurring in the Golgi region followed by appearance of the labeled protein in the plasma membrane (29). The temporal sequence of [^{3}H]fucose labeling of cones is also very similar to that found for the labeling of rhodopsin in amphibian rods with other sugars (30).

Several lines of evidence support the tentative identification of the cone specific fucosylated component as a glycoprotein, including the migration in SDS polyacrylamide gels and the staining pattern with Coomassie Blue in isoelectric focusing gels, the sensitivity of the material to pronase, and its solubility characteristics, which are most consistent with that of an integral membrane glycoprotein. No fucose has been detected in bovine rhodopsin, which does contain mannose and *N*-acetylglucosamine (7, 8, 9, 10). In the rabbit, although the disc membranes of rod OS were not labeled with [^{3}H]fucose in vivo (31), the fucosylated protein from a mixture of rod and cone OS could not be separated from rod opsin by SDS-PAGE (16). It was found (32) that antibodies to frog opsin obtained from SDS polyacrylamide electrophoresis gels cross-reacted with cone OS in sections of frog retinae, suggesting that cone OS protein(s) and rod opsin were similar in size and/or antigenicity. In the present study, by the use of goldfish retinae, which contain larger and more numerous cone photoreceptors, we have been able for the first time to detect separation of a cone-specific fucosylated protein from rod opsin, the latter of which appears quite similar to bovine opsin in mol wt and net charge. It is of interest that the goldfish cone protein is lower in mol wt than rod opsin because in the chicken, cone iodopsin also shows a lower apparent mol wt than rod opsin (R. S. Fager, personal communication.) The striking differences in net charge between the goldfish cone fucosylated protein and rod opsin was unanticipated because in the rabbit, the fucosylated cone protein and rod opsin show similar isoelectric points (16). The appearance here of 2–3 labeled bands in the isoelectric

FIGURE 6 Only the vesiculated green-sensitive cone outer segments are "stained" selectively in vitro with horseradish peroxidase, in agreement with their selective labeling in vivo with this enzyme. Note selective radioautographic labeling as in Fig. 5. Rod outer segments (*R*) are unlabeled with either marker. × 18,900.

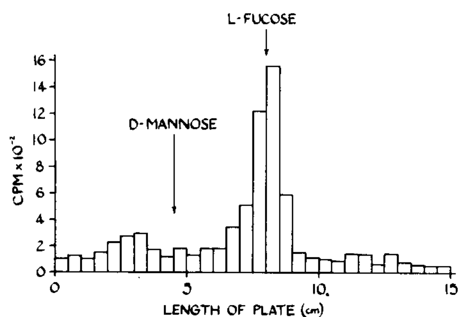

FIGURE 7 Thin-layer chromatographic separation of the neutral sugars released from [^{3}H]fucose-labeled photoreceptor membranes. Over 70% of the applied radioactivity co-chromatographed with *L-fucose,* well separated from *D-mannose,* the only neutral sugar reported in bovine rhodopsin.

focusing gels is of interest, in view of the three cone visual pigment types present in the goldfish retina (14), although we recognize that the fucosylated protein bands may very well represent electrophoretic variants of a single protein (8). The significance of the 2–3 apparently distinct species of cone glycoprotein and their possible identity as cone visual pigment(s) are areas of current investigation.

The authors are grateful to Ms. R. Patterson, Ms. L. Bredberg, Ms. I. Klock, and Mr. R. Barth for technical assistance, to Mr. J. Foltz and Mr. B. Clifton for photographic help and to Ms. J. Seng for secretarial assistance.

This study was supported in part by United States Public Health Service grants EY-01311, EY-01756, EY-02317, EY-00343, and EY-01730. Some facilities were supported in part by National Institutes of Health grant No. HD-02274.

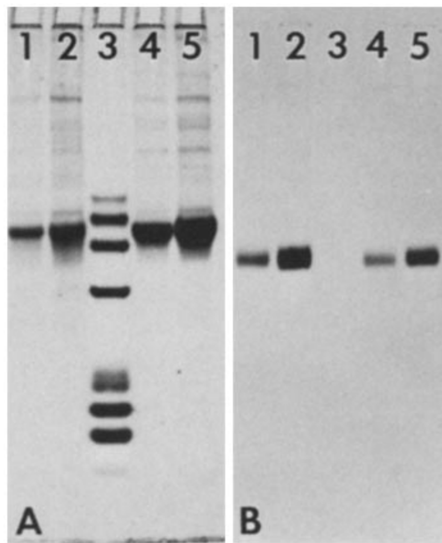

FIGURE 8 Polyacrylamide gel electrophoresis in the presence of 0.2% SDS. [^{3}H]fucose-labeled photoreceptor membranes were solubilized and applied to the gel. After staining with Coomassie Blue this gel was examined by fluorography, with an exposure of 3 d. Lane *3* contains proteins of known molecular weight: hen ovalbumin, 43,000; rabbit muscle aldolase, 40,000; rabbit muscle glyceraldehyde-3-phosphate dehydrogenase, 36,000; bovine erythrocye carbonic anhydrase, 29,000; β-lactoglobulin, 18,000; horse skeletal muscle myoglobin, 17,200; and bovine hemoglobin, 15,400 (from top to bottom). Lanes *1* and *2* contain two concentrations of photoreceptor membranes from the 1.13/1.15 g/ml interface and lanes *4* and *5* contain two concentrations of photoreceptor membranes from the 1.11/1.13 g/ml interface. *A*. Photograph of the gel stained with Coomassie Blue. *B*. Positive prepared from the fluorography x-ray film. Alignment of the x-ray film and gel was made by reference to a faint band on the x-ray film outlining the sample application cells. The major fucosylated species (33,000 mol wt) is well separated from the opsin band (38,000 mol wt).

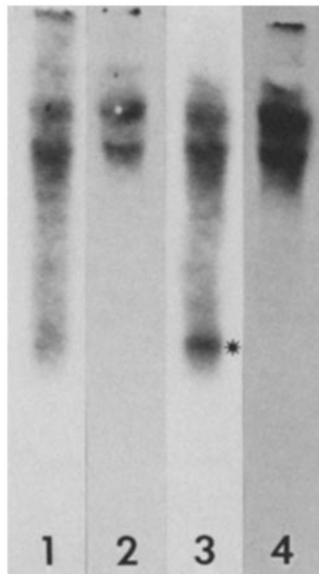

FIGURE 9 Goldfish photoreceptor membranes prelabeled in vivo with [^{3}H]fucose were solubilized in Triton X-100/urea and applied to a slab of polyacrylamide for isoelectric focusing. Fucosylated components were detected by fluorography; protein components by Coomassie Blue staining in the presence of Cu^{++}. *1*. Photograph of the Coomassie Blue-stained gel after drying onto filter paper. The sample of photoreceptor membranes was derived from the 1.13/1.15 g/ml interface of the discontinuous density gradient. *2*. Fluorogram prepared from *1*. The pI's of the radioactive components are (from the top) 8.8 (minor band), 8.6, and 8.2. *3*. As for *1*, except the sample was derived from the 1.11/1.13 g/ml interface. *4*. Fluorogram prepared from *3*. The pI's of the radioactive components are (from the top) 8.6 and 8.2. The asterisk indicates the unlabeled protein band at pI = 6.2, well separated from the radioactive components.

Received for publication 23 June 1981, and in revised form 14 September 1981.

REFERENCES

1. Maylié-Pfenninger, M. -F., and J. D. Jamieson. 1979. Distribution of cell surface saccharides on pancreatic cells. II. Lectin-labeling patterns on mature guinea pig and rat pancreatic cells. *J. Cell Biol.* 80:77–95.
2. Brabec, R. K., B. P. Peters, I. A. Bernstein, R. H. Gray, and I. J. Goldstein. 1980. Differential lectin binding to cellular membranes in the epidermis of the newborn rat. *Proc. Natl. Acad. Sci. U. S. A.* 77:477–479.
3. Nir, I., and M. O. Hall. 1979. Ultrastructural localization of lectin binding sites on the surface of retinal photoreceptors and pigment epithelium. *Exp. Eye Res.* 29:181–194.
4. McLaughlin, B. J., and J. G. Wood. 1980. The localization of concanavalin A binding sites during photoreceptor synaptogenesis in the chick retina. *Brain Res.* 119:57–71.
5. Sarthy, P. V., C. D. Bridges, D. M. K. Lam, and F. L. Kretzer. 1981. Lectin receptors on cells isolated from turtle retina. *J. Comp. Neurol.* 202:561–569.
6. Bridges, C. D. 1981. Lectin receptors of rods and cones. Visualization by fluorescent label. *Invest. Ophthalmol. Vis. Sci.* 20:8–31.
7. Heller, J., and M. A. Lawrence. 1970. Structure of the glycopeptide from bovine visual pigment 500. *Biochemistry.* 9:864–869.
8. Plantner, J. J., and E. L. Kean. 1976. Carbohydrate composition of bovine rhodopsin. *J. Biol. Chem.* 251:1548–1552.
9. Liang, C. -J., K. Yamashita, C. G. Muellenberg, H. Shichi, and A. Kobata. 1979. Structure of the carbohydrate moieties of bovine rhodopsin. *J. Biol. Chem.* 254:6414–6418.
10. Fukuda, M. N., D. S. Papermaster, and P. A. Hargrave. 1979. Rhodopsin carbohydrate. Structure of small oligosaccharides attached at two sites near the NH_2 terminus. *J. Biol. Chem.* 254:8201–8207.
11. Fager, L. Y., and Fager, R. S. 1978. Separation of rod and cone pigments from the chicken retina. *Invest. Ophthalmol. Vis. Sci.* 17 (Suppl):126.
12. Bunt, A. H., and I. B. Klock. 1980. Fine structure and radioautography of retinal cone outer segments in goldfish and carp. *Invest. Ophthalmol. Vis. Sci.* 19:707–719.
13. Bunt, A. H., and I. B. Klock. 1980. Comparative study of [^{3}H]fucose incorporation into vertebrate photoreceptor outer segments. *Vis. Res.* 20:739–747.
14. Liebman, P. A. 1972. Microspectrophotometry of photoreceptors. *In:* Handbook of Sensory Physiology, M. G. F. Fuortes, editor. Springer-Verlag, Berlin. Vol. VII/1. 481–528.
15. Papermaster, D. S., and W. J. Dreyer. 1974. Rhodopsin content in the outer segment membranes of bovine and frog retinal rods. *Biochemistry.* 13:2438–2444.
16. Saari, J. C., and A. H. Bunt. 1980. Fucosylation of rabbit photoreceptor outer segments: properties of the labeled components. *Exp. Eye Res.* 30:231–244.
17. Graham, R. C., and M. J. Karnovsky. 1966. The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney: ultrastructural cytochemistry by a new technique. *J. Histochem. Cytochem.* 14:291–302.
18. Wolfe, L. S., R. G. Senior, and N. M. K. Ng Ying Kin. 1974. The structure of oligosaccharides accumulating in the liver of G_{M1}-gangliosidosis. Type I. *J. Biol. Chem.* 249:1828–1838.
19. Fairbanks, G., T. L. Steck, and D. F. H. Wallach. 1971. Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. *Biochemistry.* 10:2606–2617.
20. Bonner, W. M., and R. A. Laskey. 1974. A film detection method for tritum-labeled proteins and nucleic acids in polyacrylamide gels. *Eur. J. Biochem.* 43:83–88.
21. O'Farrell, P. H. 1975. High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* 250:4007–4021.
22. Righetti, P. G., and J. W. Drysdale. 1974. Isoelectric focusing in gels. *J. Chromatog.* 98: 271–321.
23. Stell, W. K., and F. I. Harosi. 1976. Cone structure and visual pigment content in the retina of the goldfish. *Vis. Res.* 16:647–657.
24. Plantner, J. J., L. Poncz, and E. L. Kean. 1980. Effect of tunicamycin on the glycosylation of rhodopsin. *Arch. Biochem. Biophys.* 201:527–532.
25. Baeher, W., M. J. Devlin, and M. L. Applebury. 1979. Isolation and characterization of cGMP phosphodiesterase from bovine rod outer segments. *J. Biol. Chem.* 254:11669–11677.
26. Kuhn, H. 1980. Light- and GTP-regulated interaction of GTPase and other proteins with bovine photoreceptor membranes. *Nature (Lond.)* 283:587–589.
27. Bunt, A. H., and J. C. Saari. 1980. Differential staining of cone outer segments in vivo and in vitro. *Invest. Ophthalmol. Vis. Sci.* Suppl. 244
28. Margolis, R. K., and R. N. Margolis. 1972. Disposition of fucose in brain. *J. Neurochem.* 19:1023–1030.
29. Haddad, A., G. Bennett, and C. P. Leblond. 1977. Formation and turnover of plasma membrane glycoproteins in kidney tubules of young rats and adult mice, as shown by radioautography after an injection of [^{3}H]fucose. *Am. J. Anat.* 148:241–274.
30. Bok, D., S. F. Basinger, and M. O. Hall. 1974. Autoradiographic and radiobiochemical studies on the incorporation of [6-^{3}H]glucosamine into frog rhodopsin. *Exp. Eye Res.* 18: 225–240.
31. Bunt, A. H. 1978. Fine structure and radioautography of rabbit photoreceptor cells. *Invest. Ophthalmol. Vis. Sci.* 17:90–104.
32. Papermaster, D. S., B. G. Schneider, M. A. Zorn, and J. P. Kraehenbuhl. 1978. Immunocytochemical localization of opsin in outer segments and Golgi zones of frog photoreceptor cells. *J. Cell Biol.* 77:196–210.