

A Concentration of Fucosylated Glycoconjugates at the Base of Cone Outer Segments: Quantitative Electron Microscope Autoradiography

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Rod photoreceptors show a discrete band of labeled molecules at the outer segment base shortly after the administration of radioactive protein precursors (Young, 1967). The band signifies the insertion of radiolabeled protein, primarily opsin, into rod disc membrane (Basinger, Bok and Hall, 1976). In contrast, the autoradiographic labeling pattern in cones is characteristically diffuse (Young, 1971), although cones as well as rods are now thought to replace their disc membranes continually (Anderson, Fisher, and Steinberg, 1978). Using grain count analysis in conjunction with electron microscope autoradiography, we demonstrate for the first time that the *initial* cone outer segment (COS) labeling pattern in the retinas of cone-dominant rodents is not random or diffuse after intraocular injection of [³H]-fucose – a specific glycoprotein precursor. Thirty minutes after [³H]-fucose injection, the Golgi complex is heavily labeled in both rods and cones. At 1.5 hr, labeling first appears in the periciliary region and in the basal portion of the COS. We detected a basal concentration of fucosylated molecules up to 12 hr after injection; thereafter, we found no differential distribution of labeled product molecules in COS. In rods, we observed no band of labeled protein at any of the time points sampled. The low level of labeling in rods is uniformly distributed between the basal and apical portion of the outer segments. Although the identity of the fucosylated material remains unknown, the early labeling of the Golgi complex – followed by labeling over the periciliary region and the basal portion of the outer segment – suggests that newly synthesized membrane protein is transported from sites of synthesis in the inner segment and inserted into disc membrane at the COS base. However, in contrast to rods, the protein is apparently capable of diffusing longitudinally throughout the interconnected membrane network.

Key words: photoreceptor; cone; fucose; rod; glycoprotein.

1. Introduction

Rods and cones exhibit significant morphological and biochemical differences, most of which remain poorly understood although they almost certainly reflect the cells' specialized functions. The rate of photopigment regeneration is known to be much faster in cones than in rods (Wald, Brown and Smith, 1955; Rushton and Henry, 1968). The ultrastructural morphology of the two receptor classes is also different. In cones, many, and perhaps all, lamellae are in continuity with the enclosing plasma membrane; in rods, only the most basal discs show this property (Cohen, 1970; Laties, Bok and Liebman, 1976; Anderson et al., 1978). The most recent distinction to emerge is the demonstration that cone outer segments in many species incorporate fucose – a specific glycoprotein precursor (Bennet, LeBlond, and Haddad, 1974) and terminal sugar residue – preferentially compared with rod outer segments (ROS) (Bunt, 1978;

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Bunt and Klock, 1980). In the rabbit and goldfish retinas, this property has been used to isolate cone-specific membrane proteins that have biochemical features similar to rod opsin (Saari and Bunt, 1980; Bunt and Saari, 1982).

Another long-standing, but as yet unexplained, difference between rods and cones is related to the autoradiographic pattern of protein incorporation within their outer segments. Young (1967) showed that a discrete band of radiolabeled protein appears at the base of rod outer segments shortly after the administration of radiolabeled protein precursors. At longer intervals the band migrates away from the ROS base, indicating a progressive displacement of discs from the base of the stack toward the outer segment tip and, therefore, a continuous replacement of old discs with new ones. It soon became apparent that the band of labeling was due to the fact that membrane proteins, once inserted, are confined to individual rod discs (Basinger, Bok and Hall, 1976). In cones, a similar band of radiolabeled protein is not detected, despite substantial indirect evidence that disc membrane turnover is similar to that in rods (Anderson et al. 1978). Instead, product molecules are always described as being diffusely distributed over the outer segment regardless of the interval between incubation or injection and subsequent fixation (Young, 1971; Anderson and Fisher, 1976; Bunt, 1978). In contrast to rod discs, COSs in lower vertebrates, and possibly in mammals as well, may consist of one continuous lamellar network. Therefore, it is possible that membrane proteins undergo not only lateral diffusion within single discs, but also longitudinal diffusion throughout the entire membrane network (Cohen, 1973; Poo and Cone, 1973). In theory, this could explain the observed labeling pattern in cones.

In order to test this hypothesis, we quantified the initial labeling distribution over COSs in electron microscope autoradiograms at specific intervals after [³H]-fucose injection. Our results show that the *initial* pattern of [³H]-fucose-labeled product molecules in COSs is not random or diffuse. A significant concentration of label appears near their base 1.5 hr after injection, persists for up to 12 hr, and then becomes randomly distributed throughout the outer segment. Although the identity of the labeled cone outer segment glycoconjugate(s) remains unknown, the results are consistent with the view that newly synthesized membrane proteins are inserted into new lamellar membrane at the COS base. Thereafter, they undergo a slow longitudinal redistribution throughout the rest of the disc membrane network.

2. Materials and Methods

Animals

Two species of squirrels, the California ground squirrel (*Spermophilus beecheyi*) and the Eastern Grey Squirrel (*Sciurus carolinensis*) were used. The ground squirrels were trapped locally in Santa Barbara county, California; grey squirrels were purchased from Starling Squirrel Ranch, Noches, Texas. The retina of the California ground squirrel has been shown to contain 90–95% cones (Long and Fisher, 1985). The Grey squirrel retina is composed of approximately 60% cones and 40% rods (Cohen, 1964; Anderson and Fisher, 1976). Six ground squirrels and two grey squirrels were used in the investigation.

Intraocular injections and tissue fixation

Animals receiving an intraocular injection were first given an intramuscular injection of Ketamine HCl (0.5 ml kg⁻¹) and an ocular local anesthetic was applied (Alcaine). 0.5 mCi of [³H]-L-fucose (50 Ci mmol⁻¹; Amersham) was evaporated to dryness and redissolved in 50 μ l of balanced salt solution. It was then slowly injected into the center of the vitreous cavity using a microliter syringe fitted with a 30 gauge needle. Backflow from the *pars plana*

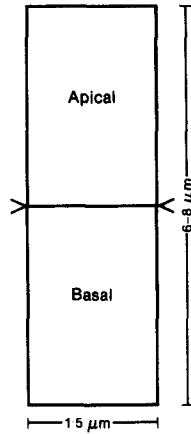


FIG. 1. Schematic diagram illustrating the method of grain-count analysis described in the text. Outer segments were partitioned into two compartments of roughly equal surface areas. Only the grains that fell within the outer segment boundaries were included in the counts. The proportion of grains μm^{-2} in the basal compartment is expressed as (basal grains per total outer segment grains).

injection site was prevented by occluding the syringe hole between the tips of forceps for about 30 sec after the needle was withdrawn. Squirrels were fixed by intracardiac perfusion of an aldehyde mixture (1% glutaraldehyde, 1% paraformaldehyde in 0.1 M phosphate buffer; pH 7.1) 0.5, 1.5, 3.0, 4.0, 8.0, 12.0, and 24 hr after injection of the labeled compound. The retinae were processed for electron microscopy using conventional methods that have been published previously (Anderson and Fisher, 1976).

Electron microscopic autoradiography

Electron microscopic autoradiograms were prepared according to the method of Young and Droz (1968). In brief, sections (60–70 nm) were placed on parlodian-coated glass slides, and stained with methanolic uranyl acetate (5 min) followed by lead citrate (10 min). A thin layer of carbon was vacuum-evaporated over the slides. The slides were then dipped under sodium vapor illumination into an aqueous dilution (1:5) of Ilford L-4 nuclear track emulsion. The slides were dried, transferred to light-tight boxes, and exposed at 4°C for 45–120 days. The autoradiograms were developed in complete darkness in Phenidon for 1 min at 15°C, stopped and fixed. Finally, the parlodian film was floated off the glass slides onto a H₂O surface, grids were placed over the sections, and the film with the grids was transferred onto filter paper. After immersion in amyl acetate to thin the parlodian layer the grids were ready for viewing in the electron microscope.

Grain count analysis (see Fig. 1)

In order to quantify the distribution of labeled molecules within the rod and cone outer segments, we devised a method of grain count analysis that gave us the percentage of silver grains in the proximal and distal one-half of each outer segment. We measured the length of well-aligned rod and cone outer segments in 8×10 enlargements of electron microscope autoradiograms. We divided the length measurement in half such that each outer segment was partitioned into two compartments (basal and apical), each having roughly equal areas. We counted only those grains falling within the outer segment boundaries in order to minimize the potential effect of crossfire from adjacent structures. Under our experimental conditions, the rod and cone outer segments are roughly equivalent to a solid band radioactive source as defined by Salpeter, Bachmann, and Salpeter (1969). Under these conditions, the HD value, i.e. the distance from the source which delimits the area containing 50% of the grains, is approximately 140 nm; this number is about six to seven times smaller than the half-width of the source. Therefore, from the standpoint of autoradiographic resolution, we were confident that the vast majority of counted grains originated from the outer segments.

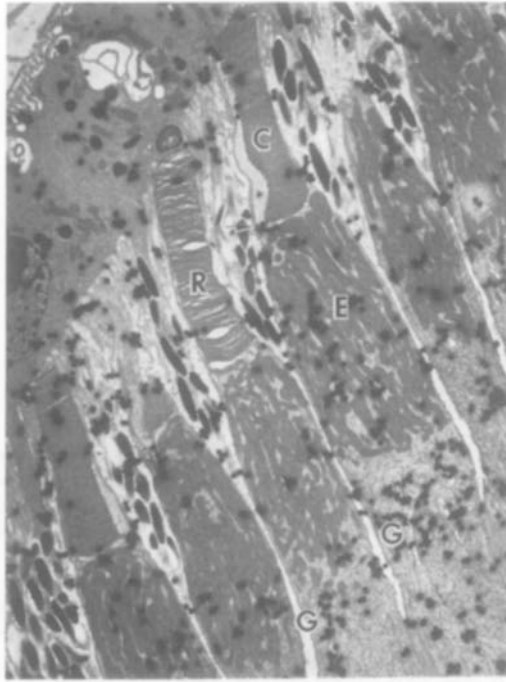


FIG. 2. Electron microscope autoradiogram 1.5 hr after [^3H]-fucose injection. A cone appears on the right and a rod is shown on the left-hand side. The Golgi complex (G) of both rod and cone myoids is heavily labeled at this time. The cone ellipsoid (E) and the periciliary region (arrow) of the cone appears to be labeled. The cone outer segment (C) is also labeled, but the rod outer segment (R) shows only a few scattered grains. $\times 9000$.

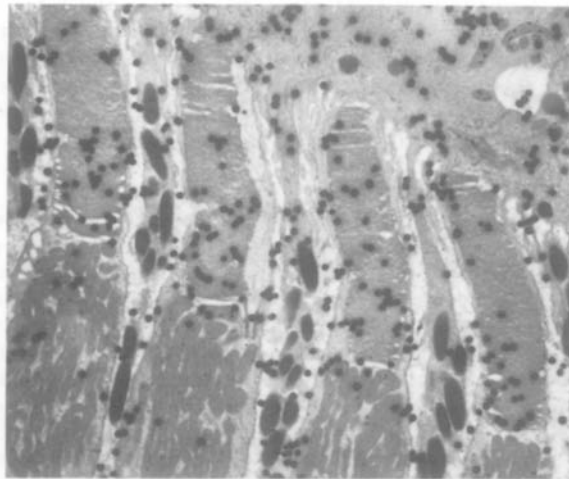


FIG. 3. Ground squirrel cone outer segments (COSs) 3 hr after [^3H]-fucose injection. At the 3 hr time-point, the basal region of these four COSs is more heavily labeled than is the apical region. The RPE cytoplasm as well as the apical microvilli are also labeled. $\times 7600$.

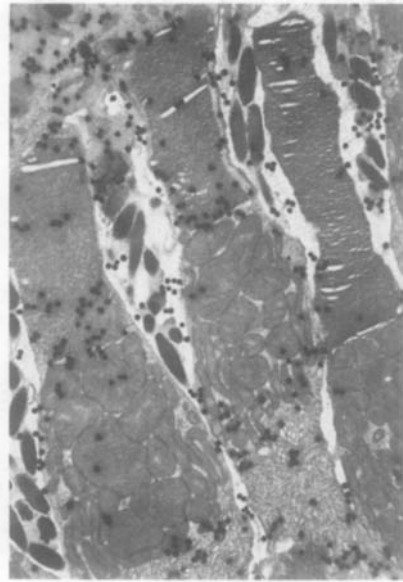


FIG. 4. Tree squirrel outer segments 4 hr after [^3H]-fucose injection. The rod outer segment (ROS) shown on the far right is very lightly labeled, while the two cone outer segments (COSs) on the left have a significant concentration of silver grains near the outer segment bases. The retinal pigment epithelium (RPE) cytoplasm (top left) and the cones' Golgi complexes are also labeled. $\times 2800$.

It is probable that a few counts originated from the thin microvillous processes that border the outer segments, or from the extracellular matrix, since these structures are immediately adjacent to the outer segments. However, both outer segment compartments are equally susceptible to this potential source of error. Similarly, a small number of grains lying outside each outer segment compartment, but possibly originating from a source within that compartment, were not counted. Using a digital image analyzer (Zeiss MOP-3), we measured the areas of both compartments, and made three calculations: (1) grains μm^{-2} for the basal compartment; (2) grains μm^{-2} for the apical compartment; and (3) total outer segment grains μm^{-2} . By taking the ratio of grains in either compartment to total grains, we obtained an estimate of the distribution of labeled molecules in the apical or basal portion of the outer segment. As few as 40 and as many as 100 outer segments were counted for each receptor type per time point combination. In order to avoid bias in selecting outer segments for grain count analysis, all aligned photoreceptors on one section of a given grid were photographed and counted. Additional non-serial grids were used as needed to obtain a minimum of 40 aligned outer segments.

3. Results

Localization of fucosylated glycoconjugates by EM autoradiography

Thirty minutes post-injection. At 30 min after injection, light microscopic autoradiograms in the ground squirrel show light diffuse labeling throughout the retina. Small clumps of silver grains are located near a few cell bodies in the nuclear layers. In the photoreceptor layer, most of the labeling appears over the rod and cone myoids – the region rich in endoplasmic reticulum that houses the Golgi complex. Small clusters of silver grains also occur within many of the myoids. The retinal pigment epithelium (RPE), the outer segments, inner segments and photoreceptor nuclei are all sparsely labeled at this time point.

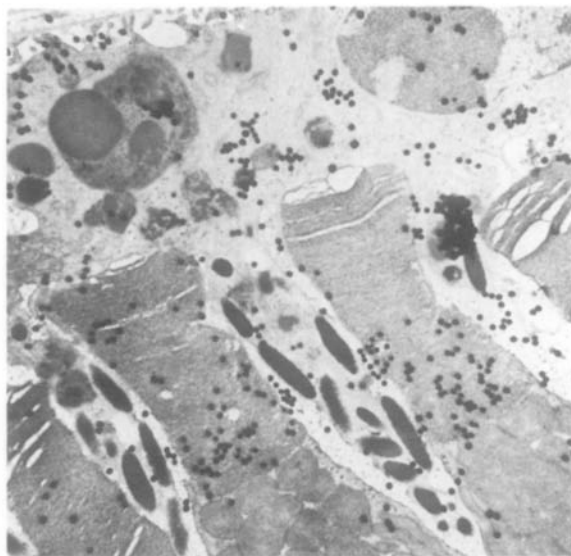


FIG. 5. Tree squirrel cones 4 hr after [^3H]-fucose injection. These cone outer segments (COS) are two examples showing a heavy concentration of silver grains near the base of the outer segments. $\times 3500$.

One and a half hours post-injection. At 1.5 hr after injection, electron microscope autoradiograms confirm that the myoid labeling in both rods and cones is due primarily to labeling of the Golgi complex (Fig. 2). The cone ellipsoids are moderately labeled, but the labeling over the rod ellipsoids is sparse. The first indication of an accumulation of silver grains in the periciliary region of the cone inner segments occurs at this time. There is also low-level labeling of COSs that appears to be restricted primarily to the basal part of the outer segment. Silver grains also occur over the RPE apical processes, or perhaps the interphotoreceptor matrix (IPM) between the microvilli, as well as over the RPE cytoplasm.

Three hours post-injection. By 3 hr after injection, the overall labeling intensity in the ground squirrel is increased and a definite concentration of silver grains is observed over the basal one-third or one-half of ground squirrel COSs (Fig. 3). The distal part of COSs is also labeled but usually not as heavily. The RPE cytoplasm, the apical processes, and the IPS are labeled above background, as are the inner segment ellipsoids.

Four hours post-injection (tree squirrel). In the tree squirrel at 4 hr, the pattern is similar to the ground squirrel at 3 hr. The Golgi complex is heavily labeled in both receptor types (Fig. 4). The cones show a distinct concentration of silver grains near the outer segment base (Fig. 5), while ROS are virtually unlabeled.

Eight hours post-injection. The overall grain density 8 hr after injection is significantly higher than at the shorter time points. The concentration over the basal COSs is clearly evident, but it is somewhat more dispersed (Fig. 6). There is also increased labeling over both the RPE microvilli and the IPM. There is no sign of a band at the base of ROS nor any significant diffuse labeling of ROS.

Twelve hours post-injection. At 12 hr after injection, all COSs remain heavily labeled. Some continue to show a distinct gradient of labeling while others do not (Fig. 7). Rods continue to show only a few scattered grains over their outer segments. Numerous

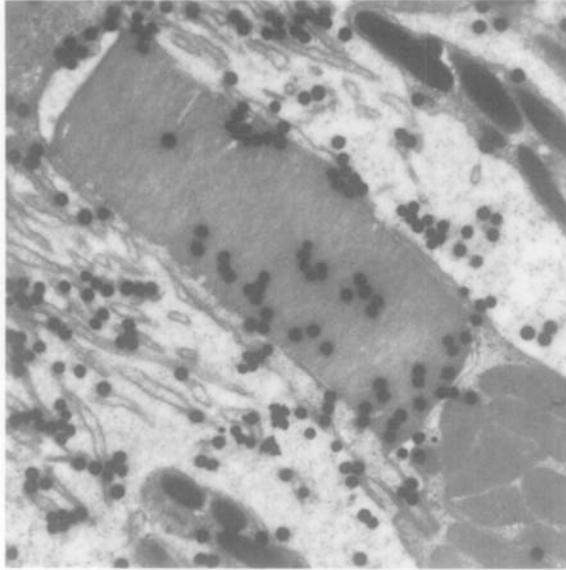


FIG. 6. Ground squirrel cone 8 hr after [^3H]-fucose injection. This cone outer segment shows a more dispersed labeling pattern, but most of the grains are still concentrated over the basal one-half of the outer segment at this time. The retinal pigment epithelium (RPE) microvilli and/or the extracellular matrix are clearly labeled by 8 hr after injection. $\times 12750$.

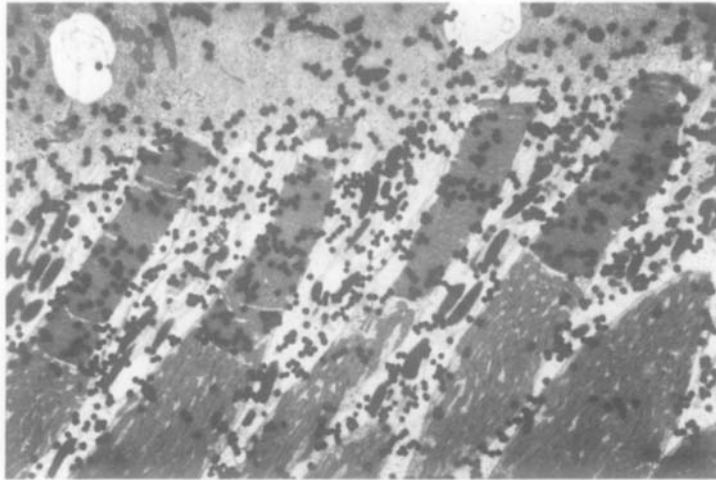


FIG. 7. Ground squirrel cones 12 hr after [^3H]-fucose injection. In this electron microscope autoradiogram, the outer segments are heavily labeled, but the basal labeling distribution is not as pronounced as it is at earlier time points. At 12 hr, the distribution tends to shift toward a more diffuse pattern; quantitatively, however, a basal concentration can still be detected in some cone outer segments. $\times 7800$.

silver grains occur over the apical microvilli as well as the basal infoldings of the RPE cells; silver grains are scattered throughout the RPE cytoplasm. Müller cell microvilli are also moderately labeled, although the resolution is not sufficient to determine whether the radioactivity is associated with the microvilli, with the extracellular matrix between the villi, or with both structures.

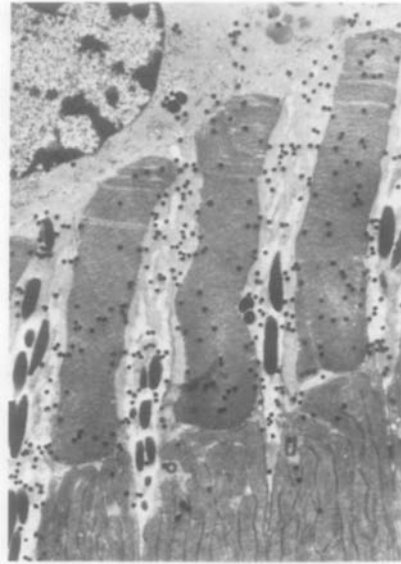


FIG. 8. Ground squirrel cones 24 hr after [^3H]-fucose injection. At the 24 hr time point the cone outer segment labeling pattern appears diffuse. $\times 7500$.

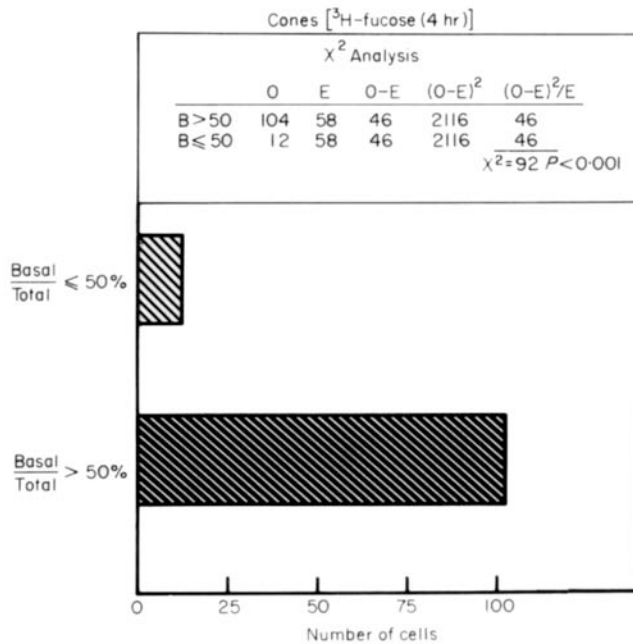


FIG. 9. Histogram showing the labeling distribution in tree squirrel cone outer segment 4 hr after [^3H]-fucose injection. COs with more than 50% of the grains in the basal compartment were compared with those that had 50% or fewer grains in the basal compartment. One hundred and four out of 116 COs showed some degree of basal labeling at this time point. A chi square analysis of this data indicates that observed distribution is significantly different from the expected distribution at well beyond the 0.001 level of significance.

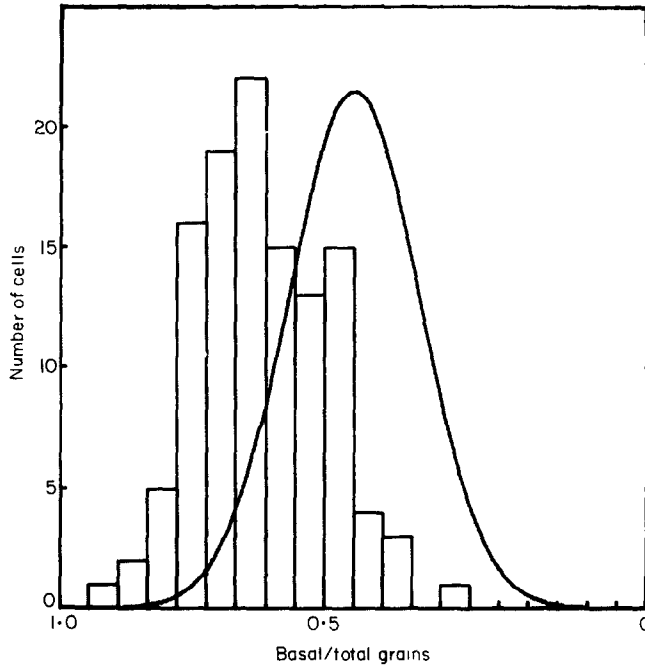


FIG. 10. Frequency histogram of [^3H]-fucose labeling in tree squirrel cone outer segments 4 hr after injection. The x-axis indicates the proportion of silver grains in the basal compartment per total outer segment grains (μm^2). The range extends from 1.0 (100% of grains in the basal compartment) to 0.0 (no grains in the basal compartment) (bin width = 0.05). The continuous curve represents a theoretical distribution for randomly labeled outer segments [the standard deviation (s) is arbitrarily equated to the sample s]. The theoretical distribution should approximate a normal distribution with most cells having about 50% of the grains in each compartment. The sample distribution, however, is skewed sharply to the left, indicating that there is a higher than expected number of cones with grains concentrated in the basal compartment of the outer segment (see Fig. 1). Total number of cones = 116, $\bar{x} = 0.61$, $s = 0.11$.

Twenty-four hours post-injection. At 24 hr, there is no qualitative indication of a concentration of fucosylated molecules at the outer segment bases (Fig. 8). The rest of the labeling pattern is similar to the 12 hr time point.

Quantitative results. When we quantified the outer segment labeling in the two species and at the different time points, we found a consistent pattern. At the earliest time point analyzed (1.5 hr) the outer segment grain density μm^{-2} is low; however 31 to 39 cones (79%) have more than 50% of the grains within the basal compartment (all counts were computed as grains μm^{-2}). At 3 hr, the outer segment grain density jumped sharply, as did the proportion of COS with more than 50% of the grains in the basal compartment (40 to 41). The mean proportion of grains in the basal compartment to total grains is 0.71 ± 0.17 ; that is, the average cone has over 70% of the total grains in the basal one-half of the outer segment. On average, each cell has well over twice as many grains basally, compared to the apical compartment.

In the grey squirrel, at 4 hr, the mean proportion of grains in the basal compartment to total grains is slightly less than in the ground squirrel at 3 hr (0.61 ± 0.11). Nevertheless, the vast majority of cones (104 to 116) have more than 50% of the grains in the basal compartment. A chi-square analysis of the data showed that this difference is significant at beyond the 0.001 level of significance (Fig. 9). A frequency histogram

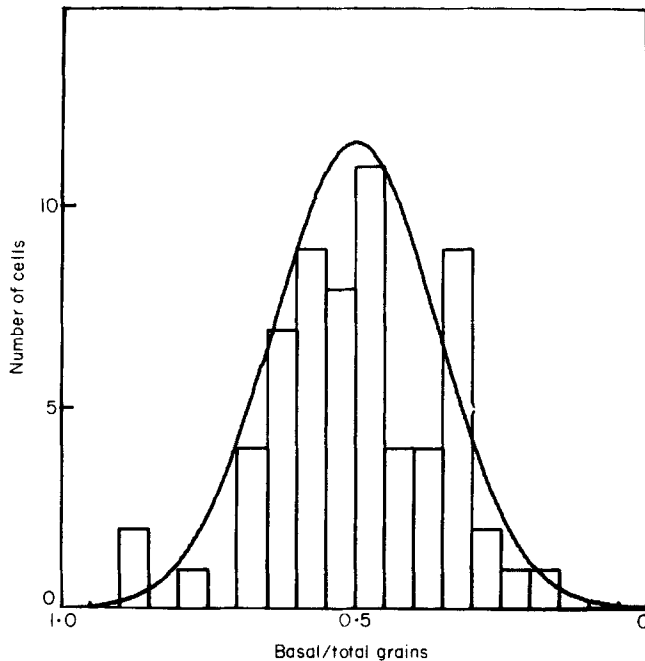


FIG. 11. Frequency histogram of [^3H]-fucose labeling in tree squirrel rod outer segments (ROSs) 4 hr after injection. A sample of 63 ROSs was analysed and the data were plotted in the same way as described in Fig. 10. In this case, there was a reasonably good fit between the sample (bars) and theoretical (continuous curve) frequency distributions. The analysis indicates that labeling in most ROSs tends to be evenly distributed between the basal and apical compartments. $x = 0.45$, $s = 0.14$.

of this data is shown in Fig. 10. The continuous curve represents a theoretical distribution for randomly or diffusely labeled outer segments with the standard deviation equated arbitrarily with the sample standard deviation. The theoretical pattern should approximate a normal distribution, with most cells having roughly equal numbers of grains in each compartment. The number of cells having either a basal or apical concentration should drop off on either side of the 0.5 value. Instead of matching the normal distribution, the sample distribution is skewed to the left, indicating that most cones at this time point have a higher than expected proportion of grains in the basal compartment.

Although the mean grain density is much lower in ROSs (cones had about twice as many grains μm^{-2} at this time point), we quantified the rod data in the same way in order to test the validity of our grain count analysis. We found, as expected, that the sample frequency distribution fit the theoretical one rather well (Fig. 11). In addition, when we plotted the rod data we confirmed that about 50% of the cells have more grains in the basal compartment and about 50% have more grains in the distal compartment (Fig. 12).

Our next step was to quantify the labeling distribution in a series of ground squirrels killed at specific times after injection. The results from this analysis are shown in a time sequence histogram (Fig. 13). On the x -axis is the proportion of grains μm^{-2} in the basal compartment to total grains, with 1.0 indicating that all of the grains fell in the basal compartment, 0.5 meaning that 50% of the grains were in the basal compartment, and so forth. On the y -axis is the number of COSSs. The results show

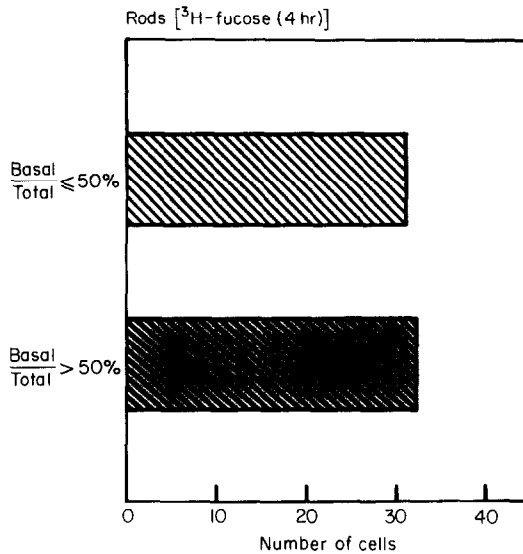


FIG. 12. Histogram showing the [³H]-fucose labeling distribution in tree squirrel ROS 4 hr after injection. In contrast to the cone data (see Fig. 9), approximately one-half of the ROS sample ($n = 63$) had more than 50% of grains in the basal one-half of the outer segment; the other half had 50% or fewer grains in that compartment. The observed distribution shows clearly that the grains are evenly distributed between the basal and apical portions of the ROSs at this time.

clearly that most COSs have a basal concentration up to about 12 hr after injection. At the 12 hr time point, the skewed distribution appears to erode, such that by 24 hr the sample approximates a normal distribution, indicating that grains in most COSs are evenly dispersed between the two compartments.

4. Discussion

[³H]-Fucose is generally regarded as a specific glycoprotein precursor (for a review, see Schachter, 1978). In brain, it is incorporated primarily into glycoprotein, and usually not into glycolipid or glycosaminoglycans (Margolis and Margolis, 1972). In retina, [³H]-fucose is also incorporated, in unmodified form, primarily into glycoprotein (Saari and Bunt, 1980; Bunt-Milam and Saari, 1982; Fliesler, Tabor and Hollyfield, 1984). The available evidence indicates that retinal glycosaminoglycans, located principally in the interphotoreceptor matrix, do not contain fucose (Berman and Bach, 1968; Bach and Berman, 1971a, b). Human donor retinas incubated in the presence of [³H]-fucose, and then analyzed by SDS-PAGE and fluorography, show a number of newly synthesized, fucosylated proteins (Fliesler et al., 1984) one of which is secreted into the incubation medium (Hollyfield et al. 1985). Grain count analysis of light microscopic autoradiograms from these retinas indicates that [³H]-fucose is incorporated predominantly into the photoreceptor layer (Fliesler et al., 1984). [³H]-Fucose has also been employed in autoradiographic studies of interphotoreceptor matrix synthesis (Feeney, 1973), and of glycoprotein synthesis in the developing retina (Blanks and Blanks, 1980).

In tissue-processing for electron microscopy, unincorporated sugars such as fucose

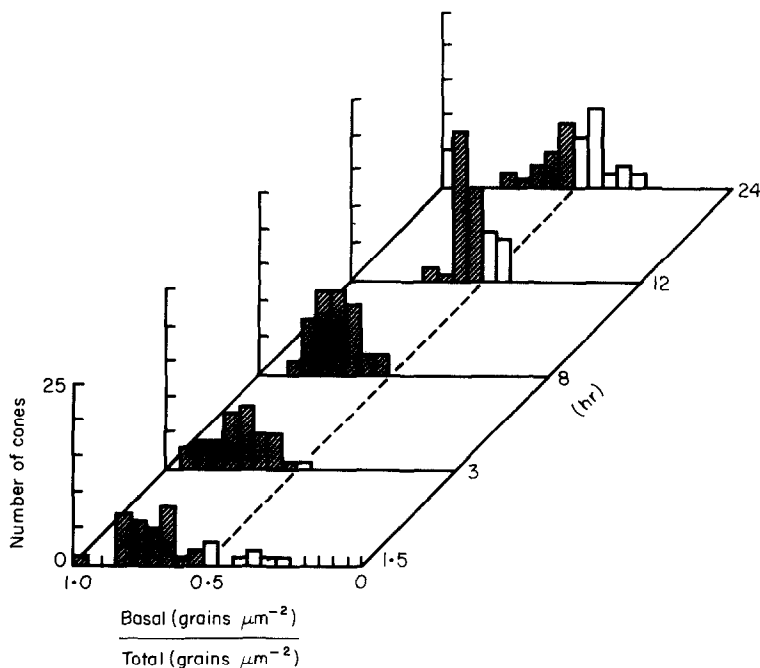


FIG. 13. Three-dimensional frequency histogram showing the extent of basal labeling in ground squirrel cone outer segments as a function of time after [^3H]-fucose injection. The proportion of basal to total grains (in μm^2) was calculated for each COS at a given time point. This is shown on the x-axis, with 1.0 indicating that 100% of the grains occupied the basal compartment, 0.5 meaning that 50% of the grains were in the basal compartment, and so forth (bin width = 0.05). All COS that had more than 50% of the grains in the basal compartment are indicated by cross-hatched bars. On the y-axis is the number of COSs; the z-axis denotes the time interval in hours after [^3H]-fucose injection. The graph shows clearly that the vast majority of COS have a basal labeling distribution up to about 12 hr after injection. Thereafter, more COS tend to have a roughly uniform labeling distribution. At 24 hr, the COSs approximate what would be expected from a random or diffuse labeling distribution (see text).

and glycolipids containing fucose residues, are not normally retained in the tissue because they tend to be extracted by the solvents employed (Bennett et al., 1974). Unlike free amino acids which may be cross-linked by glutaraldehyde, and thereby fixed in place under certain conditions (Peters and Ashley, 1967), free fucose is not known to be cross-linked by commonly used fixatives. Therefore, the prevailing evidence is fully consistent with the conclusion that the [^3H]-fucose labeled material identified in squirrel COS by autoradiography is a glycoprotein or group of glycoproteins.

The autoradiographic distribution of labeled protein in vertebrate COSs has classically been characterized as diffuse, irrespective of the time interval after fixation or the protein precursor utilized (Young, 1971). Bunt (1978), however, did suggest that the *initial* [^3H]-fucose labeling of type II or cone-like photoreceptors in the rabbit may not be completely random. Our quantitative results now confirm that observation in the cone-dominant retinas of two different mammals and suggest a specific time course for the redistribution of membrane proteins throughout the COS.

The identity of the labeled protein within the COS remains obscure, but there are two well-characterized glycoproteins that could be considered viable candidates.

Interphotoreceptor retinoid-binding protein (IRBP) is a soluble, high-molecular-weight glycoprotein of the complex type. In addition to fucose, it contains mannose and glucosamine as well as the other terminal sugars, sialic acid and galactose (Fong, Liou, Landers, Alvarez and Bridges, 1984). IRBP has been localized to the interphotoreceptor space (Bunt-Milam and Saari, 1983; Fong et al., 1984; Anderson et al., in press; Schneider and Papermaster, 1985), to small granules within the rod and cone inner segments (Bunt-Milam, Saari, and Bredberg, 1985), and to the interior of cone outer segments using immunoperoxidase techniques (Bunt-Milam and Saari, 1983). In the ground squirrel, it appears to be one of the principal fucosylated proteins in the outer retina (Anderson et al., in press). In theory, the presence of IRBP within the cone intradisc space could account for the observed fucose-labeling in squirrel COSs. Such an arrangement might facilitate the transport of retinol into and out of COSs if IRBP is, in fact, a transport protein. If the labeling in cones does represent IRBP, it could conceivably diffuse into the intradisc space of cone discs (Bunt-Milam and Saari, 1983), some or all of which are patent with the extracellular space (Cohen, 1970; Laties et al., 1976). Alternatively, it could be transported through the connecting cilium into the outer segment from sites of synthesis in the inner segment.

If IRBP is synthesized in the photoreceptor inner segments and transported to the outer segment via the connecting cilium, we should have observed significant [³H]-fucose labeling in the outer segments of both rods and cones. In the squirrel, ROS labeling is very sparse at all time-points sampled. In view of recent evidence implicating rods as the primary cell type involved in IRBP synthesis (Hollyfield et al., 1985), this interpretation seems untenable. Secondly, if IRBP simply diffuses into the cone intradisc space from the interphotoreceptor space, we should also have observed [³H]-fucose labeling over the 'open' discs at the ROS base, and yet we found no such labeling. In addition, the dimensions of the IRBP molecule may constitute a barrier to diffusion into the cone intradisc space. IRBP, with a Stoke's radius of 5.6 nm (Adler, Evans and Stafford, 1985; Bunt-Milam, Saari, Klock and Garwin, 1985), would have to diffuse into an intradisc lumen the width of which is reported to be 3.0-4.0 nm by X-ray diffraction (Blaurock and Wilkins, 1969; Corless and McCaslin, 1984).

Electron microscopic localization of IRBP using a post-embedding, immunogold procedure supports the view that IRBP is not present in rod and cone outer segments (Anderson et al., in press). Intense extracellular IRBP-labeling is associated with an electron opaque coat that closely borders the COS plasma membrane as well as the connecting cilium. Only a few patches of this opaque matrix appear adjacent to the ROS plasma membrane. No labeling is present within the outer segments of squirrel, cat, or rhesus monkey photoreceptors using this technique (Anderson et al., in press; unpubl. observations). This absence of immunocytochemical labeling must be interpreted cautiously because the lack of labeling does not necessarily indicate the absence of the antigen. Nevertheless, these results lead to the tentative conclusion that IRBP is not present within rod or cone outer segments and, therefore, could not account for the fucose-labeling observed in the COSs of different species - including the squirrel.

Opsin is the major glycoprotein of rod disc membranes, comprising approximately 80-90% of the total membrane protein (Hall, Bok and Bacharach, 1969; Heitzmann, 1972). The saccharide composition of bovine opsin is limited to mannose and *N*-acetylglucosamine residues *in vivo* (Plantner and Kean, 1976; Fukuda, Papermaster and Hargrave, 1979; Liang, Yamashita, Muellenberg, Shichi and Kobata, 1979). But

bovine opsin can accept terminal sugars such as galactose or fucose to form a modified oligosaccharide chain *in vitro* (O'Brien, 1976). Canine rhodopsin has recently been shown to contain fucose as part of its oligosaccharide chain (Aguirre and O'Brien, *in press*). The latter result increases the likelihood that the radiolabeled 'bands' observed by Bunt and Klock (1980) at the base of ROSs in other species (e.g. monkey, chicken, turtle, gecko, frog) may actually represent labeled opsin. A cone-specific protein, having biochemical properties of a membrane protein and, in the goldfish, showing a different molecular weight from rod opsin, is labeled after [³H]-fucose injection into goldfish and rabbit eyes (Saari and Bunt, 1980; Bunt and Saari, 1982). Therefore, it is possible that the fucosylated material identified in squirrel cones, and in the cones of many other species by autoradiography (Bunt and Klock, 1980), represents cone opsin.

Opsin has been localized immunocytochemically to rod disc membranes (Papermaster, Schneider, Zorn and Kraehenbuhl, 1978) and to the enclosing plasma membrane (Jan and Revel, 1974). It is capable of rapid lateral diffusion within single discs (Liebman and Entine, 1974; Poo and Cone, 1974). Autoradiographic findings strongly suggest that newly synthesized rhodopsin diffuses throughout the enclosing plasma membrane soon after insertion into ROS membranes (Basinger et al., 1976), but photobleaching recovery experiments indicate that it is incapable of diffusing longitudinally from disc to disc (Liebman and Entine, 1974; Poo and Cone, 1973, 1974). The ultrastructural basis for this conclusion rests with the fact that all but the most basal rod discs are thought to be discrete units connected to the plasma membrane and to each other only by filamentous bridges (Usukura and Yamada, 1981; Roof and Heuser, 1982), whereas most if not all cone lamellae are in direct continuity with the enclosing plasma membrane (Cohen, 1970; Laties et al., 1976). Because the COS is thought to be a continuous network, newly-inserted membrane proteins may not be restricted to lateral diffusion within individual cone lamellae. They could theoretically diffuse throughout the lamellar stack after being inserted at the outer segment base (Poo and Cone, 1973; Cohen, 1973). Liebman and Entine (1974) found no evidence for the predicted rapid longitudinal exchange of visual pigment in mudpuppy COS, and concluded that diffusion of cone visual pigment is impeded at the lamellar rims. However, Liebman (1975) later reported a slow longitudinal exchange in cones that occurred over a 20 min period.

Our autoradiographic results in cones may be related to this phenomenon. The initial labeling of the Golgi complex, followed by a marked increase of labeling in the periciliary region and over the basal discs, parallels the biosynthetic pathway established for opsin in frog rods (see Papermaster and Schneider, 1982, for review). The subsequent randomization of newly-synthesized membrane proteins within the COS membrane network apparently requires a much longer period of time (12 hr in the squirrel) than that expected from a process of simple diffusion (Liebman and Entine, 1974). This implies that some mechanism(s) – situated, perhaps, at the lamellar rims – exist to restrict rapid longitudinal diffusion of visual pigment molecules and other COS membrane proteins.

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