THE RENEWAL OF PROTEIN IN RETINAL RODS AND CONES

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ABSTRACT

The renewal of protein in retinal rods and cones has been analyzed by quantitative electron microscope radioautography in adult frogs injected with a mixture of radioactive amino acids. Protein synthesis occurs predominantly in the ergastoplasm, localized in the myoid region of the photoreceptor cells. Much of the newly formed protein next flows through the Golgi complex. In rods, a large proportion of the protein then moves past the mitochondria of the ellipsoid segment, passes through the connecting cilium into the outer segment, and is there assembled into membranous discs at the base of that structure. Discs are formed at the rate of 36 per day in red rods and 25 per day in green rods at 22.5° C ambient temperature. In cones, a small proportion of the protein is similarly displaced to the outer segment. However, no new discs are formed. Instead, the protein becomes diffusely distributed throughout the cone outer segment. Low levels of radioactivity have been detected, shortly after injection, in the mitochondria, nucleus, and synaptic bodies of rods and cones. Nevertheless, in these organelles, the renewal process also appears to involve the utilization of protein formed in the ergastoplasm of the myoid.

INTRODUCTION

The population of cells in the vertebrate retina, in common with that in other parts of the nervous system, is stable. Once cell differentiation is complete, there is no further cell division and, consequently, no replacement of damaged or senescent cells. Nevertheless, because the continued functioning of these organs is critical for survival of the organism, some sort of renewal mechanism becomes a necessity. This is particularly evident in the case of retinal rods and cones. A most remarkable specialization has made them exceptionally sensitive to light energy but has at the same time rendered them unusually vulnerable to damage from a variety of sources (6, 17, 25, 26).

In nerve tissue, in lieu of a renewal of cells, there is a renewal of cell constituents, including protein (10, 19, 36). A peculiar problem faced by neurons is that the protein synthetic machinery, localized in the perinuclear region, must supply replacement protein for cell processes which may extend for relatively enormous distances from the cell body. This replacement of protein is accomplished by a continual current of axoplasmic material which is manufactured in the cell body and then displaced along the axon to distant sites of utilization (10, 13, 35).

Due to their elongated form, retinal rods and cones face similar logistical problems of maintaining supply lines between sites of synthesis and consumption. In fact, the problem is compounded in photoreceptor cells by a remarkable segregation of organelles in different segments of the cell, by

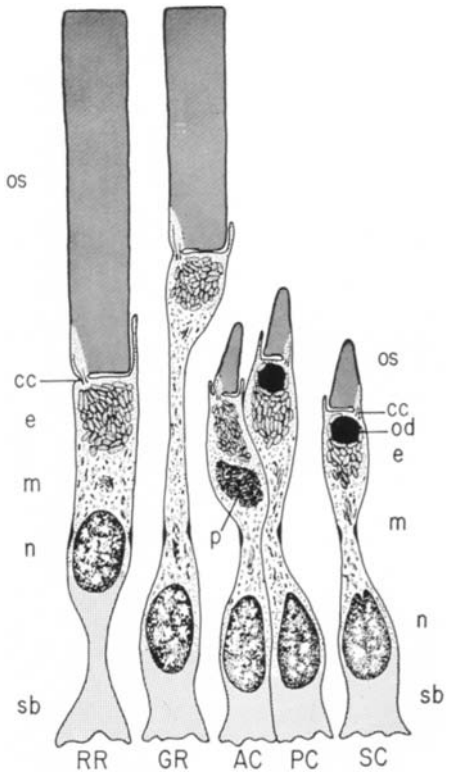

FIGURE 1 Diagram showing the two types of rods and three types of cones in the frog retina. *RR*, red rod; *GR*, green rod; *AC*, accessory cone; *PC*, principal cone; *SC*, single cone; *os*, outer segment; *cc*, connecting cilium; *e*, ellipsoid; *m*, myoid; *n*, nucleus; *sb*, synaptic body; *p*, paraboloid; *od*, oil droplet. The ellipsoid and myoid together comprise the inner segment of the cell.

a structural and functional polarization, and by an organization of the cell outer segment into a compacted pile of membranous discs (Fig. 1).

Protein is distributed throughout the photoreceptor cells and is particularly concentrated in the outer segment (7). However, the synthesis of protein is largely restricted to the inner segment, particularly its myoid portion (7, 20, 27, 38). Droz (7) presented the first evidence that renewal of protein involves an intracellular redistribution of that cell constituent. In radioautographic studies of retinal rods in rats and mice, he found that some newly formed protein remains in the inner segment, presumably serving local replacement needs, whereas a considerable portion of it migrates to the outer segment. Young (38) reported that protein delivered to the rod outer segment accumulates at the base of that structure and then gradually moves along it as a disc-shaped unit which ultimately disappears at the apical end of the cell. This observation suggested that the entire rod outer segment might be undergoing renewal by repeated addition of new membranous discs at the base of the outer segment, coupled with a balanced removal of disc material at the cell extremity (38, 39). This process may not occur in retinal cones. At least in the frog, replacement protein becomes diffusely distributed in the cone outer segments, indicating that different mechanisms of protein renewal may occur in this class of photoreceptors (40).

The following report describes a study of protein renewal in rods and cones of the frog retina analyzed by means of quantitative electron microscope radioautography after the administration of radioactive amino acids.

MATERIALS AND METHODS

Each of nine adult frogs (*Rana esculenta*), averaging 30 g body weight (range 28–33 g), received 10 mc of a tritiated amino acid solution containing equal amounts of radioactivity due to histidine, methionine, leucine, and phenylalanine.[1] Individual frogs were sacrificed at 10 and 30 min, at 1, 2, 4, and 8 hr, and at 1, 4, and 7 days after injection. The animal sacrificed at 10 min was injected by the intracardiac route, under Nembutal anesthesia. The remainder of the frogs were injected by way of the dorsal lymph sac, without anesthesia. The animals were maintained at 22.5° C under conditions of ordinary laboratory illumination. All were light-adapted at sacrifice.

The eyes were fixed for 1 hr at 4° C in a solution of 4% methanol-free formaldehyde, phosphate-buffered to pH 7.1. The anterior portion of the globe, including the lens, was then removed, and fixation continued for an additional hour at room temperature. Then the tissues were cut into smaller pieces, rinsed in buffer, postfixed in 2% osmium tetroxide in the same phosphate butter at pH 7.1, dehydrated, and embedded in Epon. The retinal fragments were cut out and reoriented, with epoxy cement, so that longitudinal sections of the photoreceptor cells could be obtained.

Silver sections which had been cut on an LKB

[1] L-histidine-^{3}H, generally labeled, specific activity 10.7 c/mmole and L-methionine-methyl-^{3}H, specific activity 0.3 c/mmole was obtained from the Commissariat à l'Énergie Atomique, Saclay, France. L-leucine-4,5-^{3}H, specific activity 5.0 c/mmole, and L-phenylalanine-^{3}H, generally labeled, specific activity 2.8 c/mmole, were obtained from New England Nuclear Corp., Boston, Mass.

ultrotome were deposited on glass microscope slides which previously had been coated with a thin layer of celloidin. The sections were stained with uranyl acetate and lead citrate, coated with carbon, and then dipped into Ilford L4 emulsion (at 40° C) diluted 1:4 with distilled water (16). The preparations were exposed in the dark under low humidity at room temperature for 1–3 months, were developed in Microdol X for 4 min at 17°C, and fixed in 30% sodium thiosulfate.

The celloidin membrane was separated from the slide by floating on water, and grids were placed over the sections. The membrane and grids were then removed from the surface of the water by adhesion to wet filter paper. After drying, the grids were detached from the membrane and placed in isoamyl acetate for 3 min to diminish the thickness of the celloidin supporting layer. The radioautographic preparations were then examined and photographed in a Siemens Elmiskop I electron microscope.

The number of radioactive atoms which disintegrates during the radioautographic exposure period is proportional to the number present at the beginning of this period. Therefore, if the efficiency with which these events are detected is maintained constant, the number of developed silver grains in the emulsion will be proportional to the amount of

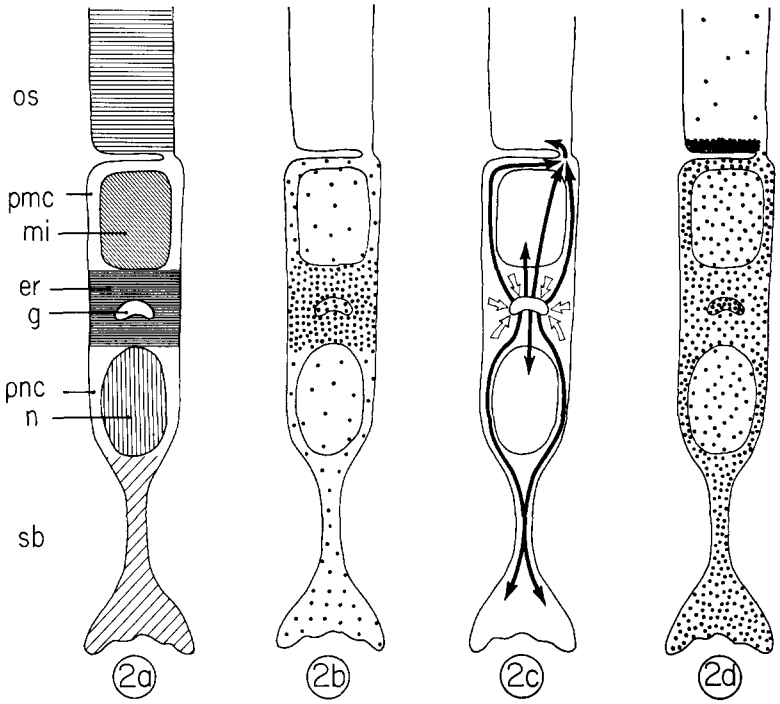

FIGURE 2 Diagrammatic representations of a red rod. The individual cell components which were analyzed separately in the quantitative studies are depicted in 2 *a*. *os*, outer segment; *pmc*, perimitochondrial cytoplasm; *mi*, mitochondria; *er*, ergatoplasm; *g*, Golgi complex; *pnc*, perinuclear cytoplasm; *n*, nucleus; *sb*, synaptic body. The distribution of protein-bound radioactivity (stippling) 10 min after injection is shown in 2 *b*. About 80% of total cell labeling is concentrated in the ergastoplasm of the myoid portion of the cell. 2 *c* illustrates the probable pathways by which this protein is redistributed within the cell. Labeled protein first moves from the ergastoplasm into the Golgi complex (white arrows). The subsequent supply lines are indicated by black arrows. A minor portion of the newly synthesized protein appears to be delivered to the mitochondria and the nucleus. A somewhat larger amount passes around the nucleus to attain the synaptic body. The greatest portion is displaced in the other direction, through the perimitochondrial cytoplasm (and perhaps to some degree between the clumped mitochondria) to the connecting structure, through which it passes to reach the outer segment. There it is largely incorporated into membranous discs at the base of that structure. The distribution of protein-bound radioactivity 8 hr after injection is shown in 2 *d*. At this interval, the concentration of labeling in the basal outer segment discs is nearly 20 times greater than that in the ergastoplasm.

radioactivity present. For quantitative evaluation, sections of comparable thickness which had been prepared, exposed, and developed together were used. Two cell classes were analyzed: (*a*) red rods and (*b*) single and principal cones (Fig. 1). Together, these comprise about 75% of all the frog retinal photoreceptors. The cells were systematically photographed at a magnification of 4,000. Prints were prepared at a final enlargement of 12,000. Individual portions of the cells (Fig. 2) were cut out of the photographs, their surface areas were estimated by weighing, and the exposed silver grains were counted. The concentration of radioactivity in each cell component was then expressed as grains per unit area (weight) at each interval studied (Tables I–III).

The relative size of the different cell components in red rods and single cones was estimated by reconstructing entire cells by the use of photomontages of low magnification photographs. The outlines of the cells were transferred to paper, and the individual components were cut out and weighed (Table IV).

RESULTS

10 min after injection, radioactivity was most heavily concentrated in the ergastoplasm (free and membrane-bound ribosomes) in the myoid portion of the frog photoreceptor cells (Figs. 2–5; Tables I–III). A weaker labeling occurred in the Golgi complex which also is located in the myoid. The mitochondrial and nuclear reactions were very low, but the thin coating of cytoplasm

TABLE I

Concentration of Radioactivity§ in Different Components of Red Rods

Cell component*	Time after injection								
	10 min	30 min	1 hr	2 hr	4 hr	8 hr	1 day	4 days	7 days
Mitochondria	1.4	1.6	2.7	5.5	7.5	7.0	7.0	5.1	5.3
Perimitochondrial cytoplasm	4.3	7.3	12.0	19.2	21.7	12.6	11.4	7.7	5.7
Ergastoplasm	19.3	18.1	17.1	15.5	16.2	13.2	11.5	7.8	6.6
Golgi complex	12.5	36.6	88.3	82.1	31.5	28.0	14.9	13.0	10.3
Nucleus	1.1	1.4	2.0	2.6	7.0	7.3	7.0	5.3	4.1
Perinuclear cytoplasm	2.2	4.3	7.0	22.6	24.7	20.0	15.5	13.1	9.5
Synaptic body	3.8	5.6	5.0	10.1	21.5	17.7	16.6	13.1	11.7
Dendrites‡	2.2	2.7	3.6	8.1	20.7	19.7	15.7	14.4	9.6

§ Silver grains per unit area.

* Concentration in outer segment given in Table III.

‡ Dendrites of horizontal and bipolar cells in synaptic body region.

TABLE II

*Concentration of Radioactivity§ in Different Components of Cones**

Cell component‡	Time after injection								
	10 min	30 min	1 hr	2 hr	4 hr	8 hr	1 day	4 days	7 days
Mitochondria	0.5	1.0	2.4	5.5	8.1	6.2	7.1	6.5	5.9
Perimitochondrial cytoplasm	2.1	2.9	7.7	9.2	13.5	9.1	9.9	7.3	5.0
Ergastoplasm	10.1	8.7	9.3	9.8	10.0	11.3	9.3	8.8	7.8
Golgi complex	0.5	3.9	28.4	44.9	26.8	26.1	19.5	8.1	10.2
Nucleus	0.5	1.4	1.8	4.2	7.9	6.7	6.9	5.0	4.9
Perinuclear cytoplasm	6.0	6.9	4.4	14.6	18.1	16.3	5.2	7.7	6.0
Synaptic body	3.7	4.9	4.4	10.9	20.5	17.6	14.7	12.5	9.4
Oil droplet	0.0	0.2	0.0	1.6	2.3	1.5	1.6	1.0	2.0

§ Silver grains per unit area.

* Single and principal cones (combined).

‡ Concentration in outer segment given in Table III.

TABLE III

Concentration of Radioactivity‖ in Rod and Cone Outer Segments

	Time after injection								
	10 min	30 min	1 hr	2 hr	4 hr	8 hr	1 day	4 days	7 days
Cones	0.0	0.3	2.5	4.6	11.1	12.6	13.6	12.8	14.3
Rods									
Apical*	0.0	0.0	0.7	1.9	2.1	2.4	3.1	2.9	1.6
Band‡	—	—	—	82.2	182.5	187.6	195.3	197.7	211.8
Basal§	—	—	—	—	—	—	68.0	29.4	22.2

‖ Silver grains per unit area.

* Region apical (scleral) to the band of intensely labeled discs.

‡ Band of intensely labeled discs.

§ Region basal (vitreal) to the band of intensely labeled discs.

TABLE IV

*Relative Size of Different Cell Components in Rods and Cones**

Cell component	Rods		Cones	
	Size units‡	Total	Size units‡	Total
		%		%
Outer segments	100.00	52	3.3	5
Mitochondria	19.4	10	12.4	19
Perimitochondrial cytoplasm	3.7	2	3.4	5
Ergastoplasm	28.6	15	11.5	18
Golgi complex	1.6	1	0.7	1
Nucleus	24.7	13	18.2	28
Perinuclear cytoplasm	2.2	1	2.7	4
Synaptic body	11.2	6	11.2	17
Oil droplet	—	—	2.2	3
Total	191.4	100	65.6	100

* Red rods compared with single cones.

‡ Arbitrary units based upon measurements of area.

surrounding these components showed a slightly higher labeling. A perceptible amount of bound radioactivity also occurred in the synaptic body. The outer segments and the cone oil droplets were unreactive.

When size differences of the corresponding cell components were taken into account (Table IV), the incorporation of labeled amino acids into rods was found to be 1.8 times greater than that incorporated into cones. In both cell classes approximately 80% of total cell labeling at 10 min occurred in the ergastoplasm of the myoid.

In rods, the concentration of radioactivity in the ergastoplasm began to decline within 30 min after injection and continued to decrease throughout the following week. In cones, ergastoplasm labeling was relatively stable, diminishing only slightly during the period of study.

In contrast, a prominent but short-lived increase of radioactivity occurred in the Golgi complex of both types of cells (Fig. 6–8). Labeling in this organelle began to rise sharply by 30 min. At 1 hr, the concentration of radioactivity in the Golgi complex in red rods was five times greater than that in the ergastoplasm. More than 15% of the total cell radioactivity was localized in the Golgi complex, an organelle representing less than 2% of the cell volume. In cones, the intensity of peak labeling in the Golgi zone at 1–2 hr was the highest attained in any part of that cell at any interval studied.

From 2 to 4 hr, labeling dropped off precipitously in this organelle in both cell types. During the following week, it further declined gradually, but significant levels of radioactivity continued to be retained within the Golgi region (Tables I, II).

At 2 hr after injection, when labeling of the Golgi complex in rods had reached a peak and was beginning to diminish, a sudden increase in radioactivity occurred at the base of the connecting cilium, a site that previously had been essentially free of labeling (Fig. 10). At this interval there were also seen, for the first time, clear traces of radioactivity in the cilium itself, in the ciliary matrix (within which the ciliary filaments are prolonged part way into the outer segment), and in the basal outer segment discs. Labeling was also observed in the region of the cilium in cones but at much lower intensities.

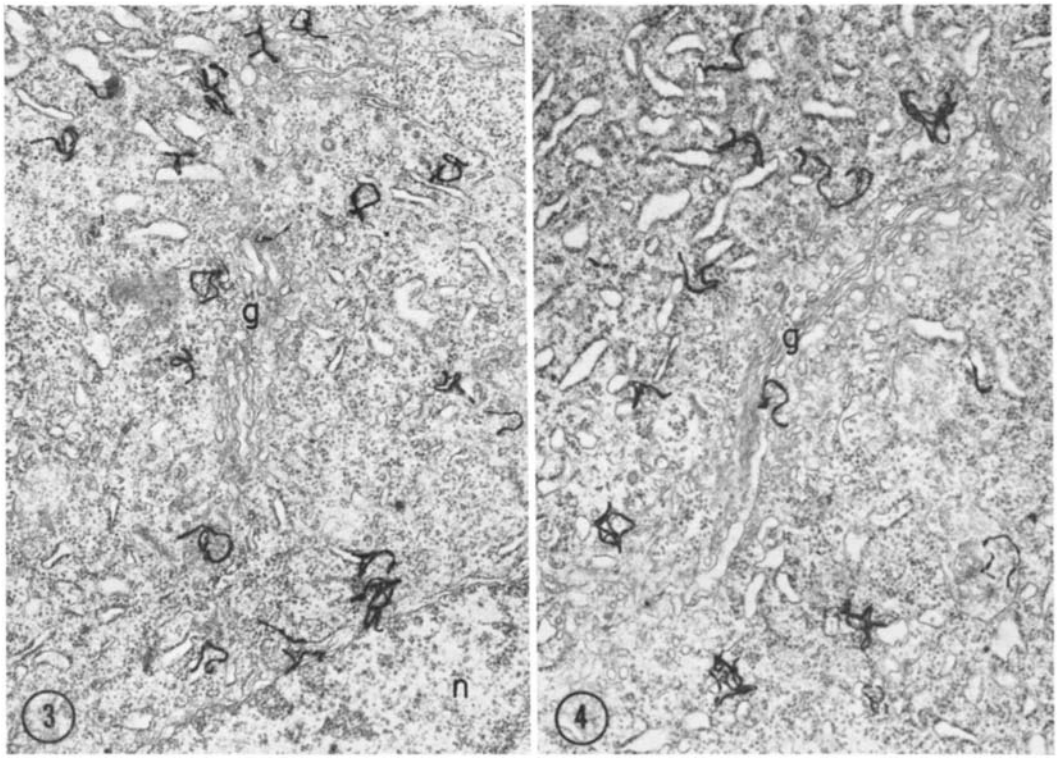

FIGURE 3 10 min after injection; red rod. Radioactivity is concentrated in the ergastoplasm of the myoid portion of the cell, the predominant site of protein synthesis. The Golgi complex (*g*) is unlabeled. A small portion of the nucleus (*n*) is visible. ×15,000.

FIGURE 4 10 min after injection; myoid of a red rod. A single silver grain lies over the Golgi apparatus (*g*). Protein-bound radioactivity is largely confined to the ergastoplasm at this interval. ×20,400.

The period 4–8 hr after injection revealed a diminished labeling in the connecting structure and a continuing increase in the concentration of labeling of the basal discs in rods (Figs. 11, 12). By then the radioactivity in these discs had reached levels unexceeded by labeling in any other structure in the retina at any interval after injection. In cones, no localization of outer segment radioactivity was observed; instead, a gradual rise in the level of scattered labeling occurred throughout the outer segment, attaining a moderate concentration which was quite stable after 8 hr (Figs. 12, 14–16). There appeared to be a similar, diffusive labeling within the outer segment of rods after 2 hr, although to a very minor degree (Fig. 15, Table III).

By 8 hr, a slight scleral displacement of the heavily reactive discs at the base of the rod outer segments could be discerned (Fig. 12); this process was obvious at 24 hr and continued during the following week (Figs. 13, 15–17). A weaker labeling was readily apparent behind the moving band. Highest immediately adjacent to the band of intense radioactivity, the reaction progressively dropped off towards the base of the outer segment (Figs. 16, 17). Thus, as the distance between the heavily labeled discs and the outer segment base increased, the "average" labeling concentration behind the band decreased (Table III).

The concentration of radioactivity in the intensely reactive discs did not diminish as they gradually were displaced apically within the outer segment. At 1 wk they were 20 times more heavily labeled than any other structure in the rod cell. By then, approximately 60% of the total radioactivity in rods was in the outer segment, much of it in the narrow band of discs in

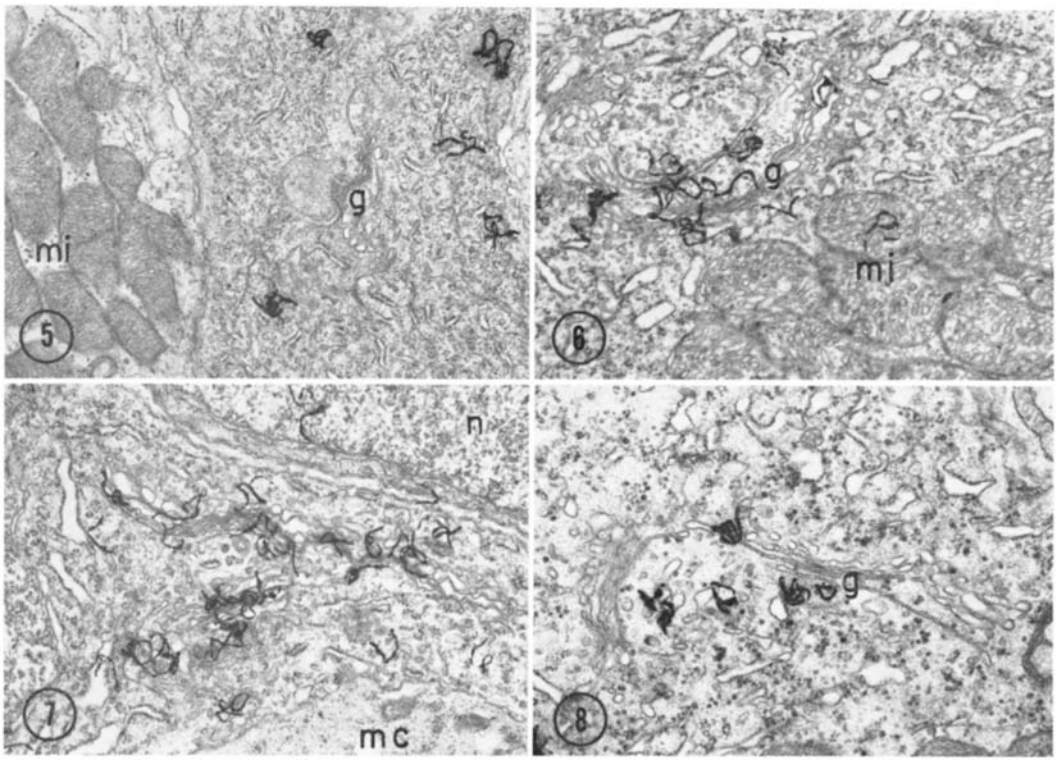

FIGURE 5 10 min after injection; myoid of a red rod (right) and ellipsoid of a principal cone (left). Labeling is restricted to the ergastoplasm. The Golgi complex (*g*) and mitochondria (*mi*) are unlabeled. ×13,200.

FIGURE 6 1 hr after injection; red rod. Protein-bound radioactivity, present initially in the ergastoplasm, has shifted into the Golgi complex (*g*), where it is now heavily concentrated. A nearby mitochondrion (*mi*) is labeled. ×17,600.

FIGURE 7 1 hr after injection; myoid of a green rod, adjacent to the nucleus. Newly formed protein has been concentrated in the Golgi complex, which is now much more heavily labeled than the adjacent ergastoplasm. *n*, nucleus of red rod; *mc*, Müller cell cytoplasm. ×15,900.

FIGURE 8 2 hr after injection; myoid of a principal cone. The kinetics of labeling are similar in cones, although the intensity of the reaction is less. At this interval, peak concentrations occur in the Golgi complex (*g*). ×19,300.

which it had been deposited during the first 8 hr after injection.

The number of discs which intervened between the base of the outer segment and the center of this band of radioactive discs was counted at 1, 4, and 7 days after injection. The resulting estimate of the rate of disc displacement was 36 discs per day in red rods and 25 per day in green rods.

The trend of labeling in the other cell components followed two general patterns, differing in the time at which peak concentrations of radioactivity were attained (Tables I, II).

In the perimitochondrial and perinuclear cytoplasm, after the low initial incorporation of radioactivity, there was a rapid rise in radioactivity at 1–2 hr which reached a peak 2–4 hr after injection (Fig. 9). Thereafter, the labeling concentration dropped rather steeply, generally falling to half the peak level by 1–4 days and approaching the initial value by 1 wk. Labeling in these regions was higher in rods than in cones.

In the mitochondria, nucleus, and synaptic body, peak concentrations of radioactivity occurred later, at 4–8 hr, with the greatest increase

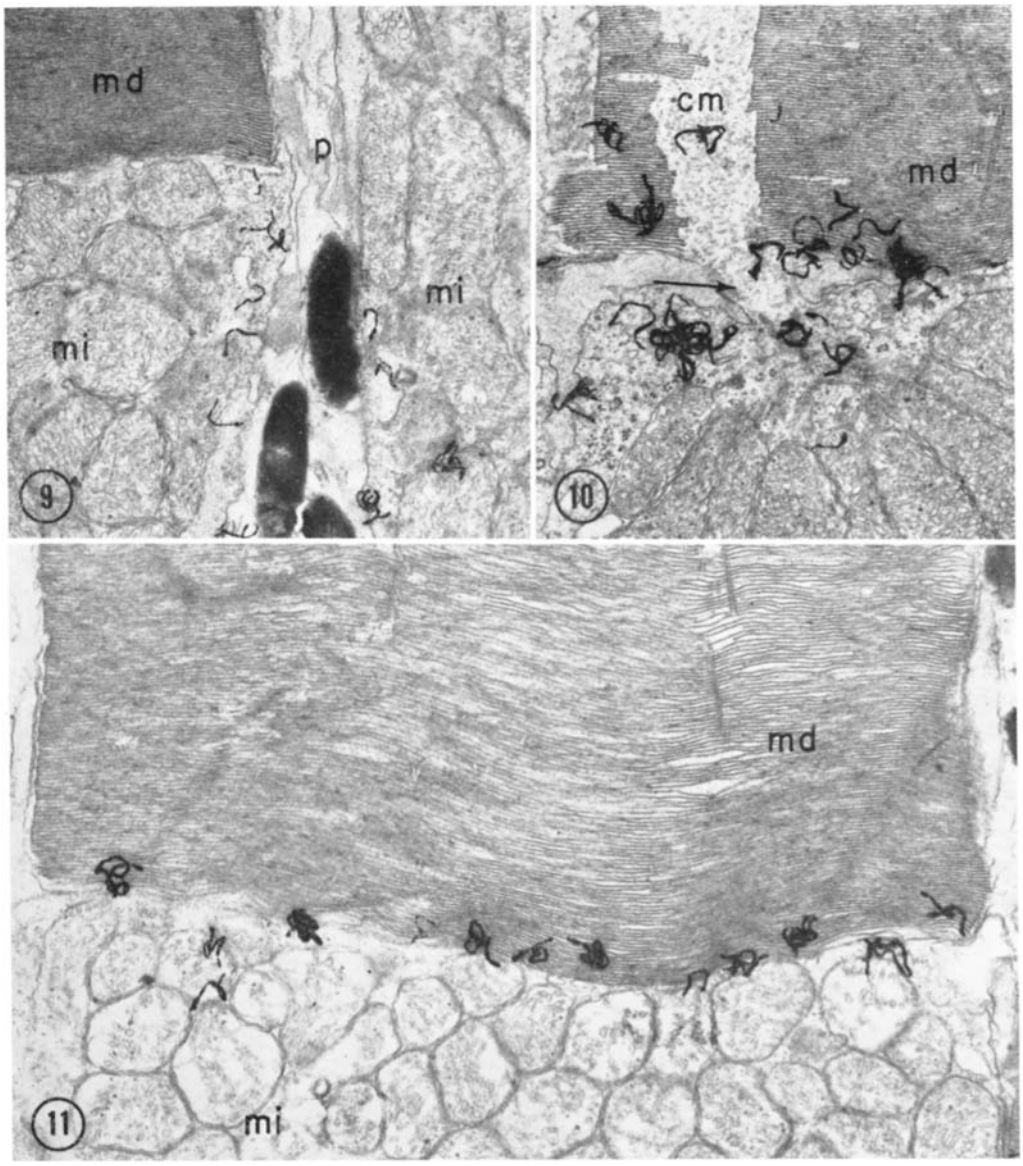

FIGURE 9 2 hr after injection; edge of the ellipsoid in two red rods. Much of the protein which is displaced to the outer segment from its site of synthesis in the myoid travels by way of the perimitochondrial cytoplasm, which is heavily labeled at this interval. *mi*, mitochondria; *md*, membranous discs at the base of the outer segment; *p*, processes of the pigment epithelium, containing pigment granules. ×16,500.

FIGURE 10 2 hr after injection; connecting cilium of a red rod. Newly formed protein attains the outer segment by passing through the connecting cilium (arrow). Accumulations of radioactivity at the base of the cilium suggest that movement of the protein is slowed at this narrow constriction of the cell. The labeled molecules circulate briefly in the ciliary matrix (*cm*), then are bound to membranous discs (*md*) at the base of the outer segment. Note the orientation of mitochondria about the base of the cilium. ×17,300.

FIGURE 11 4 hr after injection; junction of inner and outer segments in a red rod. The section does not pass through the connecting cilium. *md*, membranous discs in the outer segment; *mi*, mitochondria in the ellipsoid. Recently synthesized protein has been concentrated in the discs situated at the base of the outer segment. This is considered to be strong evidence of a continued formation of new outer segment discs at this site. ×12,400.

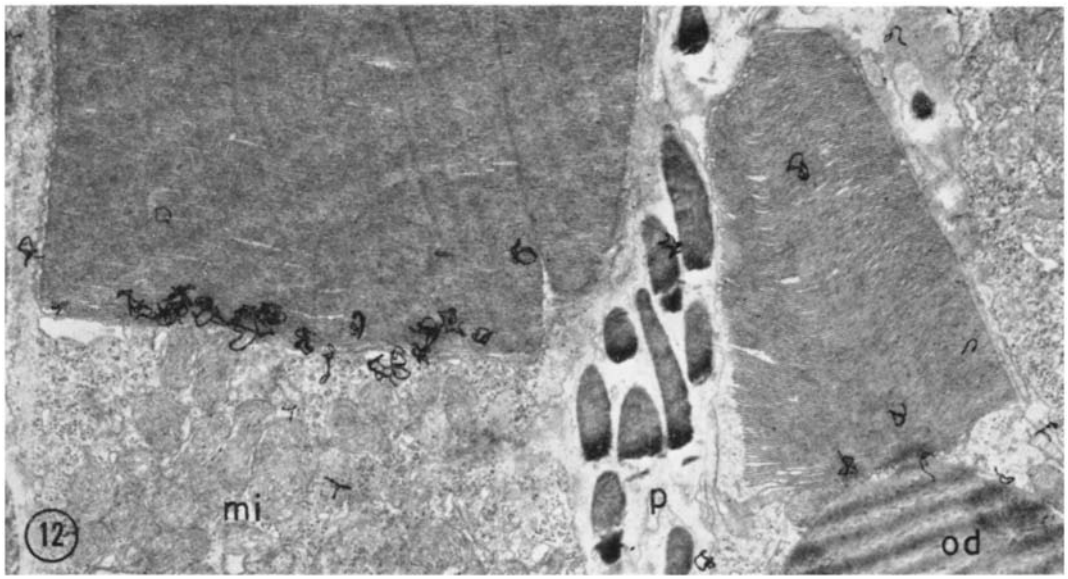

FIGURE 12 8 hr after injection. The heavily labeled discs are slightly displaced apically from the base of the outer segment in the red rod (left). In the single cone (right), no comparable concentration of radioactive protein has occurred. *mi*, mitochondria in the rod ellipsoid; *od*, oil droplet (the striation is a sectioning artifact); *p*, pigment epithelium process containing pigment granules. ×10,500.

at 2–4 hr (or slightly earlier in mitochondria). Labeling was rather low in the mitochondria and nucleus but reached a high intensity in the synaptic body (Figs. 2, 11–13, 15–18). Decline from the maximum level was gradual, approaching half the peak value at 1 wk. Labeling of these three cell components was similar in rods and cones.

Labeling in the cone oil droplets was insignificant (Figs. 12, 14, 15).

A sequence and intensity of labeling comparable to that in the rod and cone synaptic bodies occurred in the adjoining dendrites of the horizontal and bipolar cells (Fig. 18; Table I).

DISCUSSION

A mixture of radioactive amino acids was injected into adult frogs, and the fate of the labeled molecules was followed as a function of time in retinal rods and cones. Protein molecules in the process of being synthesized became radioactive upon incorporation of the labeled amino acids. Artifactual retention of free amino acids in the tissue was minimized by the use of formaldehyde fixative (2, 28) which rendered the protein products insoluble. Therefore, the first radioautographic reactions which appeared in the cells revealed the sites of protein synthesis. The subsequent appearance of labeling int sructures that initially showed little or no radioactivity was due to transfer of protein from the sites of synthesis.

Analysis of protein metabolism by means of electron microscope radioautography in a variety of cell types uniformly has shown that protein synthesis is mainly localized in the ergastoplasm, and that much of the newly formed protein then flows through the Golgi complex before being stored, secreted, or displaced to another part of the cell (3, 4, 8, 10, 14, 29, 31–34, 37). The early stages of protein renewal in these retinal rods and cones followed a similar course. Protein synthesis was concentrated in the ergastoplasm, which is located in the myoid segment of the photoreceptors. Peak ergastoplasm labeling occurred at 10 min. 20 min later, a shift of labeled protein into the Golgi complex was unmistakable.

Very high intensities of radioactivity were observed in the Golgi complex 1–2 hr after injection, indicating that a large proportion of the protein synthesized in the ergastoplasm was funneled through the smaller volume of Golgi or-

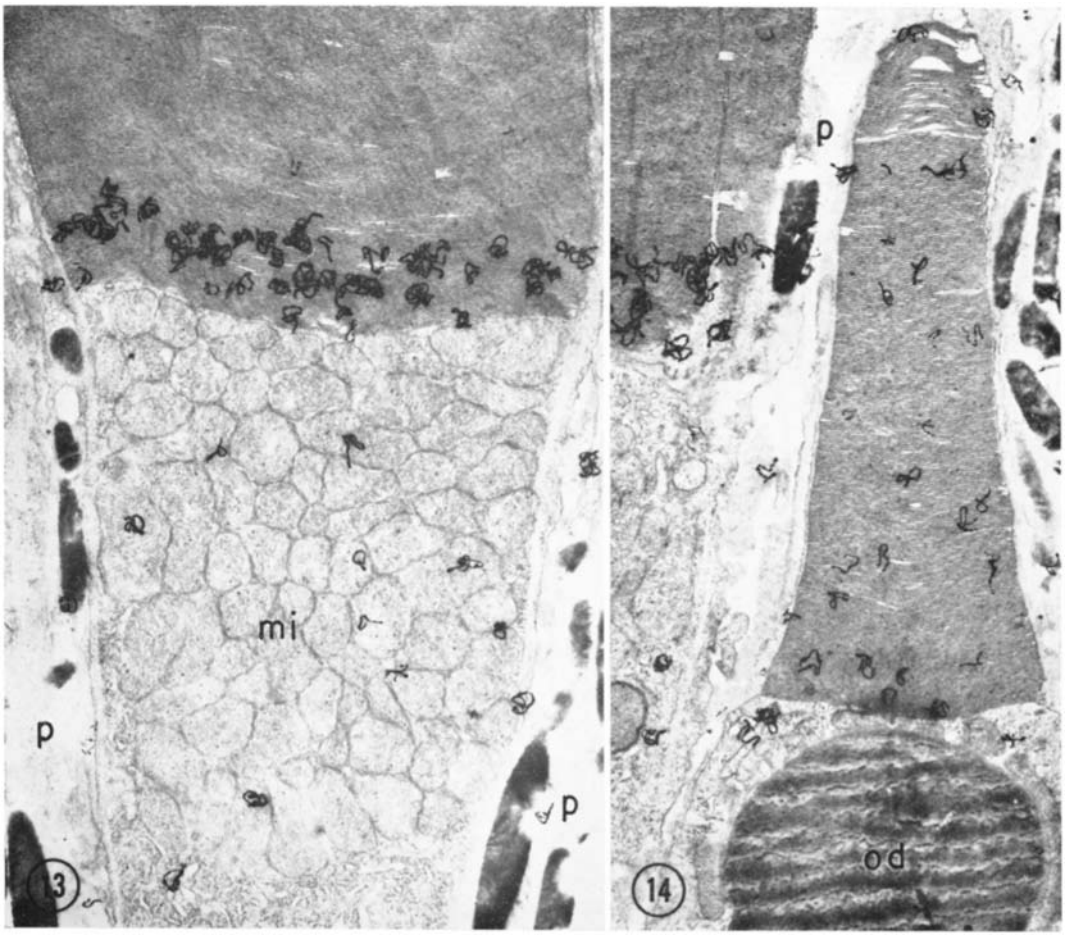

FIGURE 13 1 day after injection; red rod. The discs in which the radioactive protein has been incorporated are further displaced from the base of the outer segment. This is believed to result from the continued production of new discs in this region. There is a weak labeling of mitochondria (*mi*) in the ellipsoid zone; some of this reaction could be due to protein moving between these closely packed organelles towards the outer segment. *p*, processes of the pigment epithelium. ×10,500.

FIGURE 14 1 day after injection. Outer segment of a single cone. Protein delivered to the cone outer segment from its site of synthesis in the myoid has become diffusely distributed in that structure. There is no evidence of the neoformation of membranous discs. The cone oil droplet (*od*) is unlabeled. *p*, processes of the pigment epithelium. Part of a red rod is visible at the left. ×10,000.

ganelle. It could not be determined whether all protein synthesized in the myoid passed through the Golgi complex, since ergastoplasm labeling continued to be present beyond the time that protein began to move from the Golgi region into other parts of the cell. (There was no evidence to suggest that protein which had formed in cell structures other than those in the myoid subsequently passed through the Golgi complex.)

It is likely that the protein is modified in some way during its detour through the Golgi complex. In secretory cells, this organelle may "package" the cell product into granules or vesicles. In other cases, probably including the rods and cones, the protein may be complexed with other substances, such as carbohydrate, in the Golgi apparatus (1, 5, 9, 22, 23, 30). The prolonged retention of appreciable protein radioactivity in the ergasto-

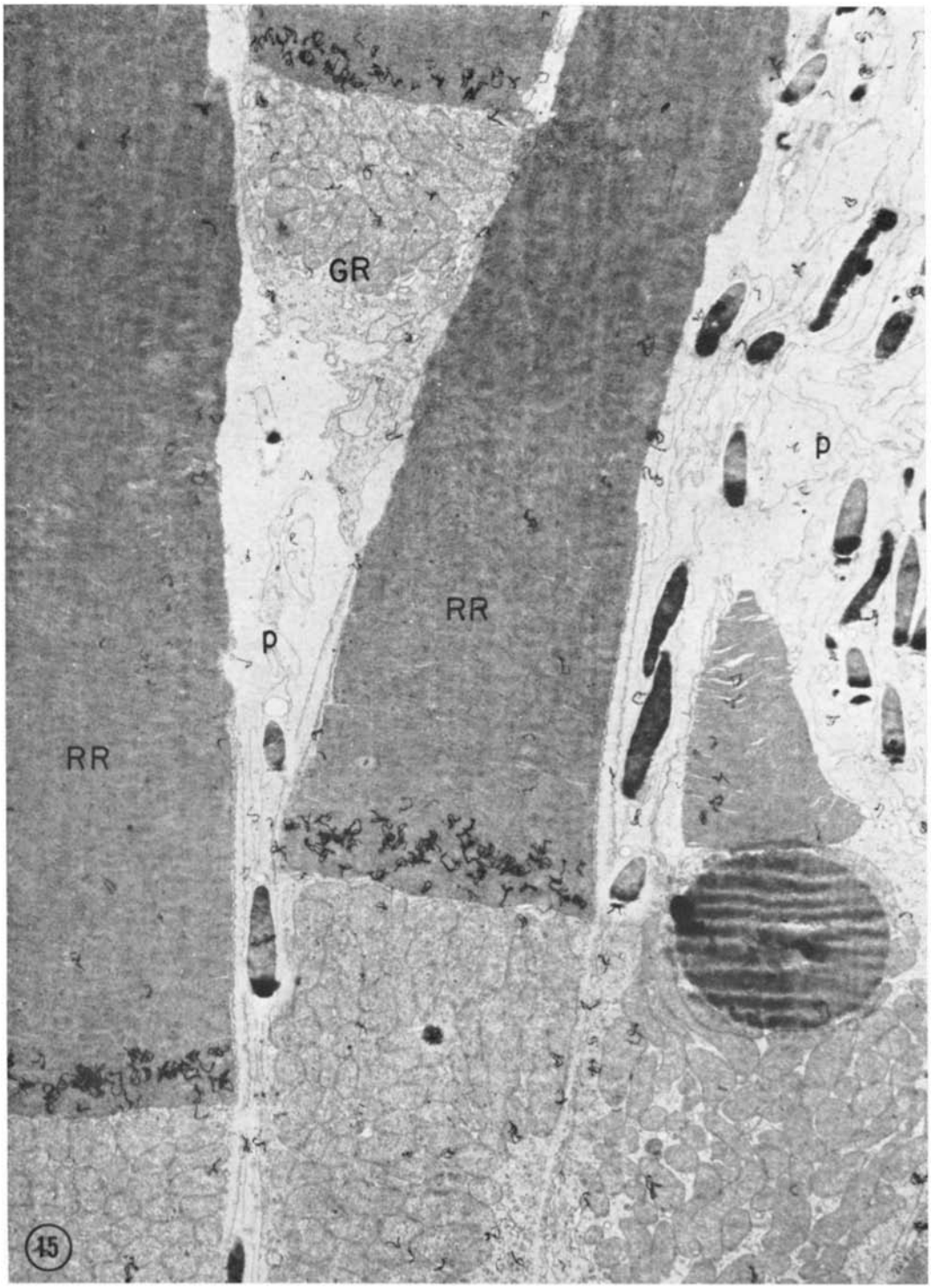

FIGURE 15 1 day after injection. Portions of two red rods (*RR*), a green rod (*GR*), and a single cone (right) are shown. In both types of rods, a group of heavily labeled discs is slightly displaced from the base of the outer segment. No such localization of protein-bound radioactivity is seen in the cone. *p*, processes of the pigment epithelium. ×6,650.

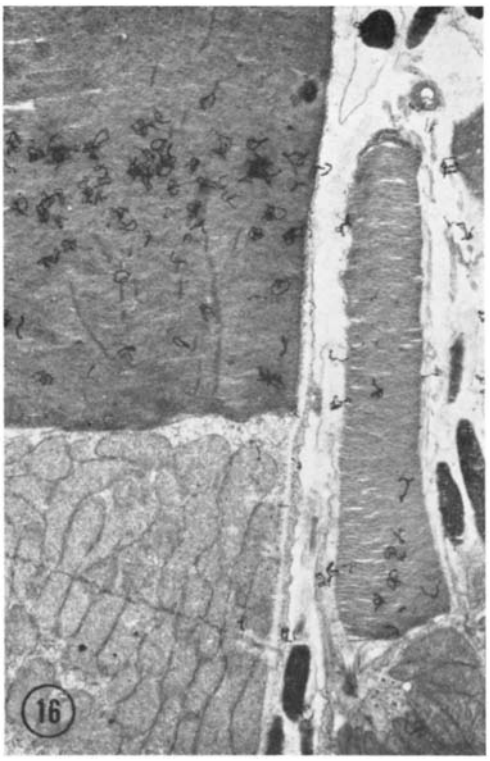

FIGURE 16 4 days after injection. The intensely radioactive discs in the red rod (left) have been significantly displaced apically by the formation of newer discs which are themselves less heavily labeled. In the accessory cone (right), only a relatively weak, diffuse distribution of radioactive protein is observed. ×7,000.

plasm and Golgi complex indicates that some of the newly formed protein may participate in the renewal of these structures.

From 2 to 4 hr after injection, labeling in the Golgi complex dropped off dramatically as the main mass of radioactive protein was displaced to other cell components. At this time, newly formed protein destined for the outer segment began to appear at the ciliated connecting structure. The transient rise of labeling in the perimitochondrial cytoplasm, peaking 2–4 hr after injection, is believed to record the passage of protein along a supply line between the myoid and outer segment (Fig. 2). The orientation of mitochondria about the base of the cilium (Fig. 10; reference 41) suggests that some protein may reach that structure by passing between the mitochondria.

The connecting cilium in frog rods and cones (and in other visual cells) lacks the two central filaments characteristic of motile cilia, and it contains an amorphous material, the ciliary matrix, which extends part way into the outer segment (41). In rods, labeled protein circulated within this matrix but was bound almost entirely to the lamellar discs at the base of the outer segment. In cones, labeled protein became distributed diffusely throughout the outer segment. Since it is improbable that these macromolecules migrated *through* the discs (38), it is likely that they penetrated the interdisc space from the side, possibly attaining the space from the ciliary matrix. This same phenomenon appears to occur also in rods, although at a very low rate.

The massive incorporation of newly synthesized protein into the basal discs of mature rod outer segments is believed to reflect the repeated formation of these membranous elements (38). This

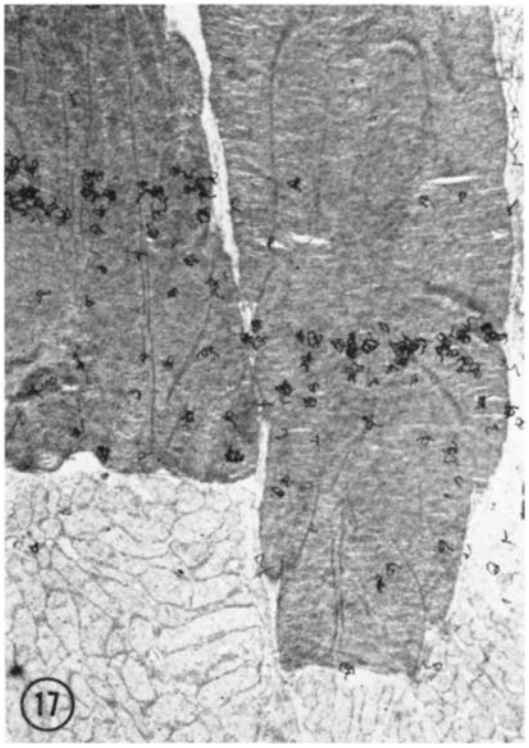

FIGURE 17 1 wk after injection; red rods. The discs containing high concentrations of radioactivity have continued their migration towards the apical extremity of the outer segment at a rate of approximately 36 discs per day. ×4,800.

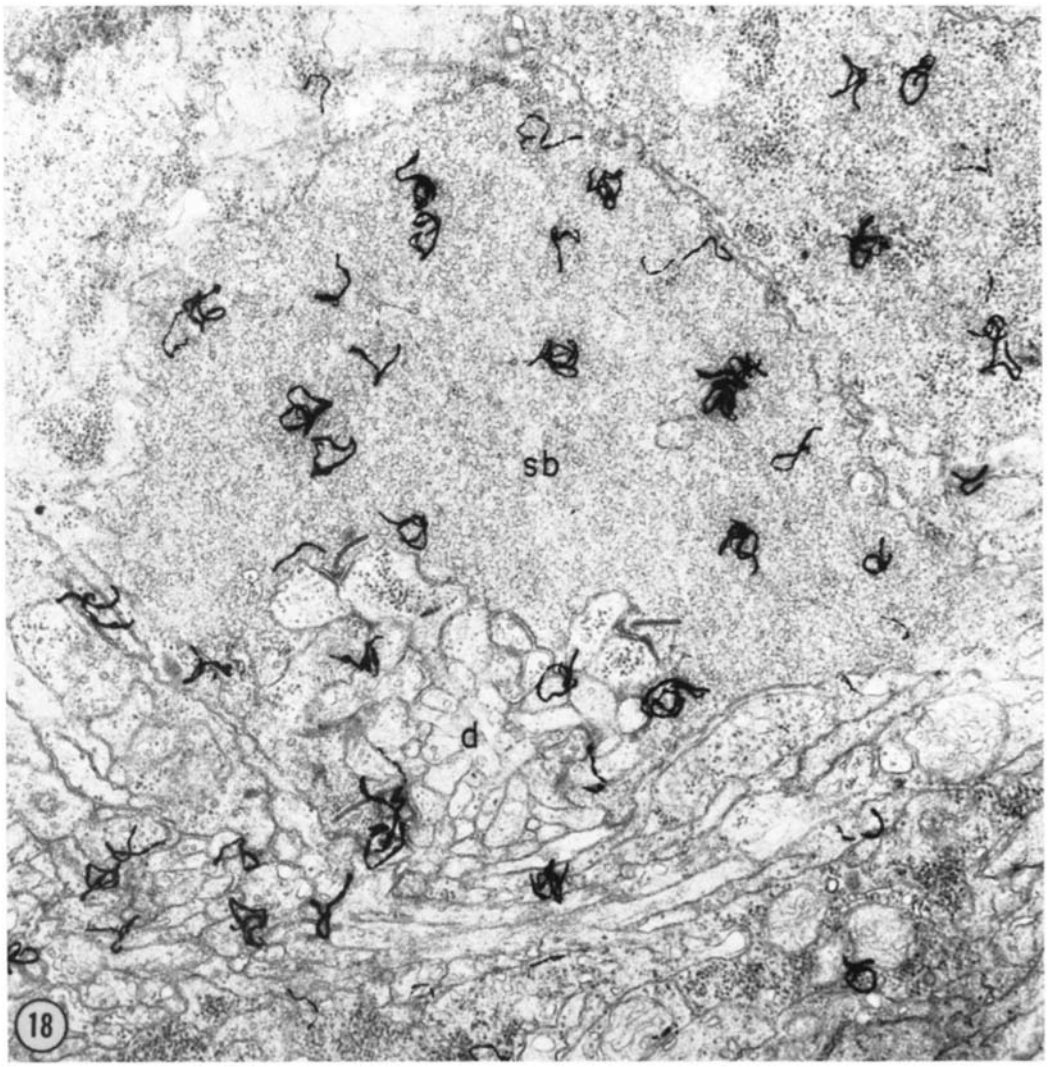

FIGURE 18 8 hr after injection; synaptic body (*sb*) of a cone. The synaptic vesicles, very weakly labeled shortly after injection, now contain high concentrations of radioactive protein, evidently due to transfer from the myoid zone of the inner segment. The adjacent dendrites (*d*) of bipolar and horizontal cells show a similar delayed increase in radioactivity, resulting from the influx of protein produced in another compartment of these cells. ×19,300.

would represent a continuation of the developmental process (24). In frog cones, for reasons not yet apparent, the production of discs ceases.

Visual pigment (rhodopsin) is considered to be an integral component of the rod outer segment disc. It has now been demonstrated that 7 days after labeled amino acid injection part of the radioactive outer segment protein in frog rods consists of visual pigment.[2] This strengthens the conclusion that the membranous discs are undergoing renewal.

Intact membranous elements have not been observed passing through the cilium. Conse-

[2] Hall, M. O., D. Bok, and A. D. E. Bacharach. 1968. Visual pigment renewal in the mature frog retina. *Science*. In press.

quently, it appears that the assembly of rod outer segment membranes occurs at the base of the outer segment and uses macromolecules which had been synthesized elsewhere in the cell.

New discs continually being produced at the base of the rod outer segments soon displaced the heavily labeled discs apically, towards the pigment epithelium. The diffuse, progressively weaker labeling behind this small group of discs resulted from formation of newer discs during a period when the specific activity of available amino acids was progressively falling (38).

Repetitive addition of new discs must be accompanied by the balanced destruction of disc material elsewhere. This destruction apparently occurs at the apical extremity of the outer segment, because the moving band of radioactivity disappears when it reaches this location (38). Probably the pigment epithelial cells, whose processes envelop the rod and cone outer segments, actively participate in the disposal of this outer segment material (38, 39). Since discs are produced at different rates in red and green rods and are not produced at all in cones, the pigment epithelial cells, if they do indeed remove outer segment material, must be able to accommodate for these differences.

A relatively low rate of protein synthesis in the mitochondria, nuclei, and synaptic bodies of these rods and cones was implied by the presence of bound radioactivity in these structures 10 min after injection. A low level of protein synthesis in mitochondria has been observed before (11, 18). Similarly, the existence of nuclear protein synthesis is well documented (10, 21). However, the early presence of bound amino acid radioactivity in the synaptic body is surprising in view of the fact that this part of the cell is filled with synaptic vesicles and is apparently free of ribosomes.

In addition to this minor *in situ* incorporation of radioactivity, the quantitative kinetic analysis revealed a gradual rise of labeling in the mitochondria, nucleus, and synaptic body between 1 and 4 hr after injection. This suggests that the major protein renewal process in these structures involves transfer of protein from synthetic sites elsewhere in the cell, presumably the ergastoplasm of the myoid. Labeling drops sharply in the Golgi complex at 2–4 hr, indicating a rapid decline in availability of labeled precursor. Since this is the interval when labeling in the mitochondria, nucleus, and synaptic body tends to increase most rapidly, the alternative explanation—that this rise is due to a slow rate of synthesis in these structures, rather than transfer—is rendered unlikely.

The observation that the source of mitochondrial protein may be both intra- and extramitochondrial is consistent with other work which seems to indicate a low capacity for protein synthesis in mitochondria but an inability to synthesize all the specific mitochondrial proteins (18).

A gradual rise in nuclear labeling has previously been noted in neurons (10). Possibly the movement of cytoplasmic protein into the nucleus is related to the phenomenon of migrating nuclear proteins observed in ameba (15).

Apparently, protein flows from the myoid around the nucleus to reach the synaptic body (Fig. 2). The rise in labeling of the perinuclear cytoplasm during the period when radioactive protein was arriving in the synaptic body, and following the peaks in the ergastoplasm and Golgi complex, is consistent with this conclusion. Protein renewal in the photoreceptor cell synaptic body has now been confirmed in the chick (12). Comparable observations made on the nerve supply to intrinsic eye muscles indicate that protein in the presynaptic terminals is renewed by transfer along the axon (12). The present work also reveals the renewal of protein in the postsynaptic terminals which are in contact with the rod and cone synaptic bodies. The rise of labeling in these neurites between 1 and 4 hr after injection bears evidence of the phenomenon of "dendritic flow" in retinal bipolar and horizontal cells.

The time sequence and intensity of labeling in mitochondria, nucleus, and synaptic body was similar in both rods and cones. The demands for protein renewal in these three organelles evidently are similar in both cell types. Since the source of most of the renewal protein is the myoid egrastoplasm, and since labeling here in cones is initially only half that in rods, a relatively higher proportion of the myoid protein output must be directed to these structures in cones, leaving relatively little protein for the outer segment, as was indeed observed.

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