THE RENEWAL OF PHOTORECEPTOR CELL OUTER SEGMENTS

RICHARD W. YOUNG

From the Department of Anatomy, and the Jules Stein Eye Institute, University of California at Los Angeles School of Medicine, Los Angeles

ABSTRACT

The utilization of methionine-^{3}H by retinal photoreceptor cells has been studied by radioautographic technique in the rat, mouse, and frog. In all three species, the labeled amino acid is concentrated initially in the inner segment of the cell. Within 24 hr, the radioactive material is displaced to the base of the outer segment, where it accumulates as a distinct reaction band. The reaction band then gradually moves along the outer segment and ultimately disappears at the apex of the cell, which is in contact with the retinal pigment epithelium. These findings are interpreted to indicate that the photoreceptor cell outer segment is continually renewed, by the repeated lamellar apposition of material (membranous discs) at the base of the outer segment, in conjunction with a balanced removal of material at its apex. The outer segment renewal rate is accelerated in frogs when ambient temperature is raised, and is elevated in both frogs and rats when the intensity of retinal illumination is increased.

INTRODUCTION

The photoreceptor cells of the vertebrate retina, the rods and cones, are highly specialized cells which respond to the stimulus of light, and transmit this response to adjoining neurons for ultimate relay to the visual centers of the brain. These functional requirements have been met through an elaborate, segmental organization, involving a remarkable degree of intracellular compartmentalization (Fig. 1).

The outer segment of the cell is comprised of a stack of many hundreds of densely packed discs, each of which represents a double layer of infolded plasma membrane. The light-sensitive visual pigment (a protein, opsin, combined with a carotenoid, retinene) is restricted to this portion of the cell, as a constituent of the discs. Outer and inner segments are continuous through an extremely narrow connecting structure, a cylindrical stalk containing a modified cilium. Ellipsoid and myoid regions may be distinguished within the inner segment. The ellipsoid is characterized by a dense aggregation of mitochondria, whereas the myoid contains the Golgi complex, scattered vesicular components, and considerable quantities of free and membrane-bound ribosomes. The nucleus is located in a widened part of the fiber, an axon-like extension which terminates in a synaptic body in association with second-order neurons.

As a result of this unusually segmented arrangement, sites of synthesis tend to be localized, and separated from sites of utilization (29). For example, the mucopolysaccharide in which the outer segments are embedded is synthesized in the inner segment. From here it is displaced in a scleral direction, into the extracellular space (13, 20). Most of the cell's ribonucleic acid is concentrated in the myoid region of the inner segment. However, the source of this material is the cell

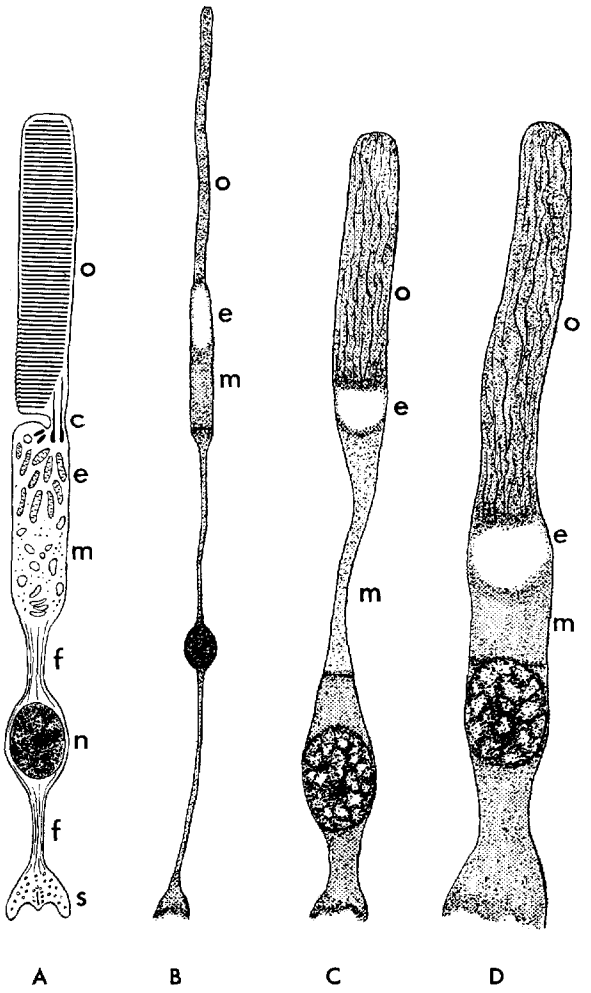


FIGURE 1 Diagrammatic representation of retinal photoreceptor cells. A is a schematic drawing of a vertebrate photoreceptor, showing the compartmentalization of organelles as revealed by the electron microscope. The following subdivisions of the cell may be distinguished: outer segment (*o*); connecting structure (*c*); ellipsoid (*e*) and myoid (*m*), which comprise the inner segment; fiber (*f*); nucleus (*n*); and synaptic body (*s*). The scleral (apical) end of the cell is at the top in this diagram. B depicts a photoreceptor cell (rod) from the rat. Mouse photoreceptors are basically similar. C is a green rod, and D a red rod, from the frog retina.

nucleus, which continually supplies newly synthesized RNA to the inner segment (2, 29).

The rapid incorporation of radioactive amino acids into the inner segment indicates that protein synthesis is confined largely to this RNA-rich compartment of the cell (8, 14, 19). Droz observed by radioautography that in rat and mouse photoreceptors most of the radioactive protein was displaced from the inner to the outer segments (8). Within a week, it apparently had become distributed evenly in this part of the cell. It was concluded that "a protein which may be opsin is continuously being exported from the inner to the outer segment of retinal rods in rats and mice" (8).

The initial results of a comparable radioautographic study in rats substantiated Droz's general findings, but differed to the extent that an alternative interpretation of the renewal phenomenon seemed to be required (27, 28). A report of this investigation, which has been expanded to include variables of species, age, temperature, and illumination, is given below.

MATERIALS AND METHODS

Tritiated methionine, in aqueous solution (specific activity 125–300 mc/mmole), was injected intraperitoneally into rats and mice, and into the dorsal lymph sac in frogs, at a dosage of 10 μc/g body weight.

In one group of experiments, animals were maintained at 25° C under conditions of 14 hr illumination (about 40 foot-candles) and 10 hr darkness per day. 29 female rats (Long-Evans) in three groups, 1, 4, and 8 wk of age at the beginning of the experiments, were sacrificed between ½ hr and 14 days

after injection. Three additional 8 wk old rats were given two injections, 2 and 6 days before sacrifice. In comparable studies, 14 8-wk old female mice (C57BL/CJ inbred strain and albinos from a closed colony of Swiss-Webster origin) were sacrificed 1 hr–10 days after injection. 31 adult frogs (*Rana pipiens*), unselected as to sex, were analyzed at post-injection intervals ranging from 1 hr to 45 days.

Frogs were used in experiments designed to explore the effects of temperature, since these animals are poikilothermic. Groups of frogs (two to three per group) were maintained at 4°, 13°, 25°, or 34° C, under the lighting conditions cited above. After 3 days in these environments, the frogs were injected with methionine-^{3}H. All were then kept at these temperatures until sacrifice 10 days later.

Additional experiments were devised to test the effects of illumination at a temperature of 25° C. Eight rats, 10 wk of age, and 10 adult frogs were studied. Half the animals were kept in total darkness.[1] The others were exposed to continuous illumination at high intensity (approximately 600 foot-candles). This was achieved by maintaining the animals in clear, acrylic plastic cages, surrounded on three sides by banks of three, 20 watt, "cool-white" fluorescent lamps. Plate glass, 6.5 mm thick, was interposed between the light source and the cage, to filter out heat and ultra-violet wavelengths. The rats were exposed to these conditions for 8 days, then injected with methionine-^{3}H, and were sacrificed after 7 additional days in this environment. The frogs were acclimated for 2 days before injection, and were sacrificed 10 days later.

At sacrifice, the eyes were removed and immediately fixed without further dissection. (Frogs were dark-adapted for 2 hr prior to sacrifice, in order to cause retraction of the retinal epithelial pigment granules which surround the photoreceptor outer segments in the light-adapted state). In the initial studies (most of the rat experiments), the eyes were fixed for 2 days in 4% buffered, neutral formaldehyde. Freeze-substitution subsequently proved to be superior, and was employed in the later experiments. The eyes were quick-frozen at −160° C in isopentane which had been cooled with liquid nitrogen. They then were stored at −80° C in a 5% solution of picric acid in ethanol for 1–2 wk. The solutions were then brought to 4° C, and the tissues transferred to absolute ethanol for 1 hr at this temperature. After an additional hour in absolute ethanol at room temperature, the eyes were embedded routinely in paraffin, sectioned at 5 μ, and prepared for radioautography with Kodak NTB2 liquid emulsion (Eastman Kodak Co., Rochester, N. Y.) by the dipping technique. The preparations were exposed for 4–16 wk, then developed in Kodak Dektol for 2 min at 17° C. The sections were stained with periodic acid–Schiff (PAS) reaction before dipping, and with hematoxylin after development.

[1] The frogs were exposed to low intensity illumination from a dim, red, photographic safelight during a daily, 1 hr feeding period.

RESULTS

Rats

In the 7 day old rat retina, the photoreceptor cells are still in the process of development. The outer segments, which begin to form about 10 days after birth, attain their mature length between 4 and 6 wk of age.

In rats injected on the 7th postnatal day, there was relatively little utilization of methionine-H^3 by the developing photoreceptor cells. However, in the 4-wk-old rats, a significant incorporation occurred in the rod inner segments, where it was slightly greater in the myoid zone. Within 8 hr after injection, labeled material had shifted perceptibly toward the junction of the inner and outer segments. By 24 hr it was concentrated as a distinct "reaction band" at the base of the outer segment. During the next few days, the band was displaced progressively in a scleral direction. After 1 wk, the band of radioactivity was situated near the apical end of the outer segments. At the next interval studied, 2 wk after injection, there was no evidence of a reaction band over the photoreceptor cells. Only a weak, diffuse labeling remained.

½ hr after the injection of methionine-^{3}H into 8-wk-old rats, a distinct radioautographic reaction appeared over the rod inner segments (Fig. 2). The reaction was slightly more intense in the myoid region. At 4 hr the ellipsoids were as heavily reactive as the myoids; at 8 hr the radioactivity was accumulating at the base of the outer segments. 1 day after injection, a distinct reaction band was visible in this region. During the following week, the band was displaced gradually along the outer segments (Figs. 3–6). After 9 days it reached the ends of the cells, which are in contact with the retinal pigment epithelium (Fig. 7). The following day it disappeared.

In both the 4 and 8 wk series, the reaction band neither increased in width nor decreased in intensity during its migration along the outer segments. There was a weak labeling of the outer segments immediately "behind" the moving band, but the portion of the outer segments in

advance of the band was unreactive above background.

In the 8-wk-old rats given two, spaced injections of methionine-^{3}H, two reaction bands were observed (Fig. 8).

Mice

The findings in 8-wk-old mice did not differ significantly from those in 8-wk-old rats (Figs. 9–11). A reaction band formed at the base of the outer segments during the first day, gradually moved along the outer segments, and disappeared at the interface with the pigment epithelium about 10 days after injection.

Frogs

In the frog retina, both rod and cone types of photoreceptor cells are present. There are two varieties of rods (Figs. 1, 12). The outer segment of green rods is relatively short, and the inner segment long, whereas in red rods, which are in the majority, the situation is reversed. Because of the relatively large diameter of the frog rod outer segments (6–8 μ), it is possible to follow the displacement of radioactivity within individual photoreceptor cells in this species.

1 hr after injection of methionine-^{3}H in adult frogs, radioactivity was concentrated heavily in the myoid region of both types of rods (Figs. 13, 14). Within 2 hr, radioactive material had begun to appear at the junction of the inner and outer segments. Most of the remaining radioactivity shifted from the myoid to the base of the outer segment within 24 hr, where it formed a distinct reaction band (Fig. 15). The band was situated at a "higher" (more scleral) level in green rods, due to the shorter length of the outer segment in these cells. Furthermore, in this cell type the formation of the reaction band lagged slightly behind that in red rods. As a result, a definite scleral migration of the band was apparent less than 2 days after injection in red rods, whereas in green rods it was 2–3 days before such displacement could be confirmed with certainty.

During subsequent days, the discrete, radioactive band gradually moved toward the apical end of the cells (Figs. 16–20). As in the rats and mice, the band neither increased in width nor decreased

---

FIGURES 2–7 Radioautographs of rat photoreceptor cells from animals 8 wk old at the time of injection of methionine-^{3}H. The pigment epithelium has been displaced artifactually from the apical end of the rods (except in Fig. 7), which is common with formaldehyde fixation. Traces of pigment granules which have remained associated with the ends of the photoreceptors are seen in Figs. 4 and 5, top left. The photoreceptor cell nuclei are at the bottom of the field. Fixation in 4% formaldehyde, pH 7; stained with PAS-hematoxylin. × 1,150.

FIGURE 2 ½ hr after injection. Radioactivity is concentrated in the inner segments of the retinal rods.

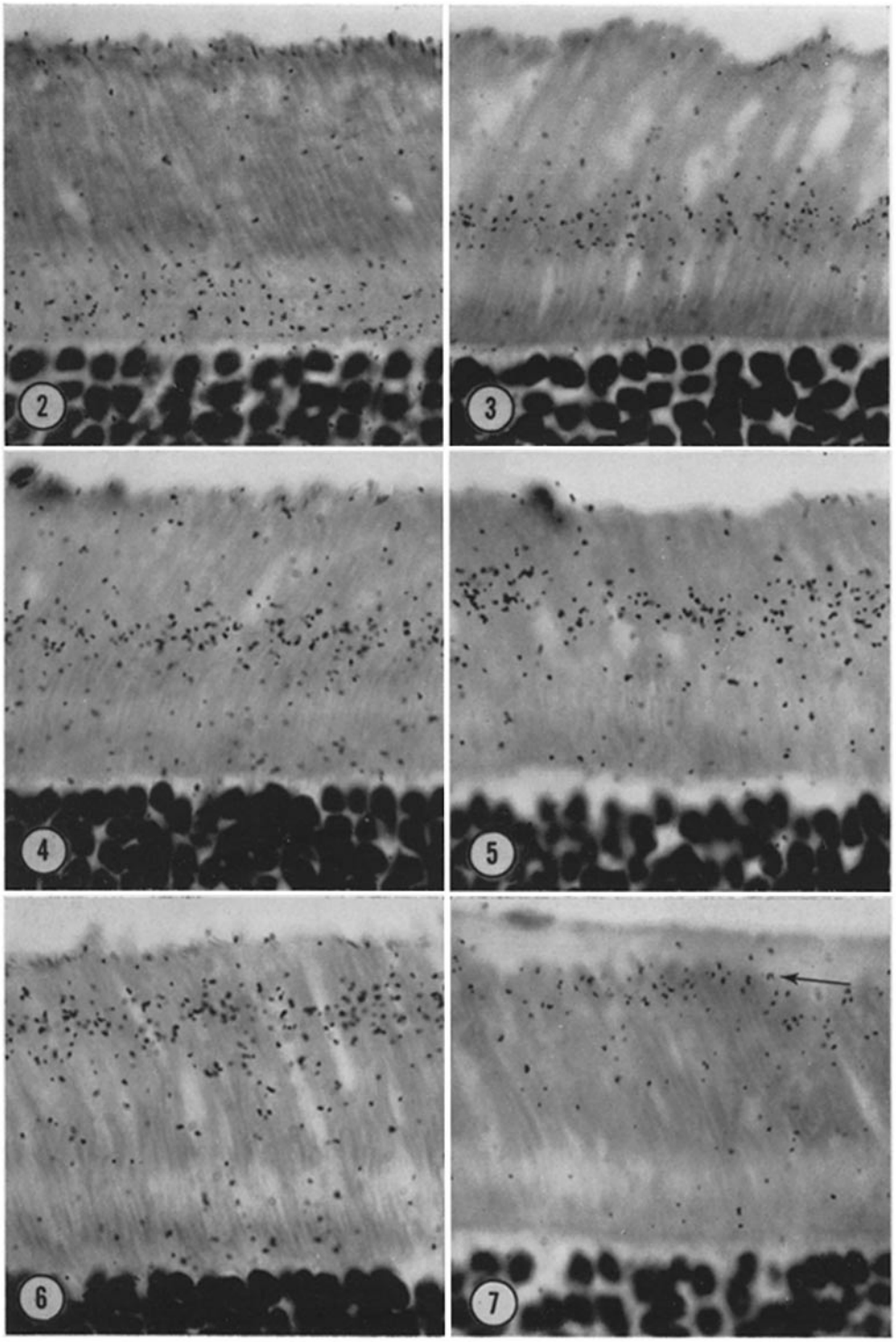
FIGURE 3 2 days after injection. The radioactive material has been displaced from the inner segment in the form of a reaction band, which is now located near the base of the outer segment.

FIGURE 4 3 days after injection. The reaction band has been further displaced.

FIGURE 5 4 days after injection. The band of radioactive material is near the midpoint of the rod outer segments at this interval.

FIGURE 6 1 wk after injection. The reaction band has neither increased in width nor decreased in intensity during its displacement. It now is located near the apical end of the outer segments.

FIGURE 7 9 days after injection. The reaction band has reached the end of the photoreceptor cells, and is disappearing at the interface with the pigment epithelium (arrow). (An albino animal was used in this experiment, so that the reduced silver grains could be distinguished more easily).


2
3
4
5
6
7

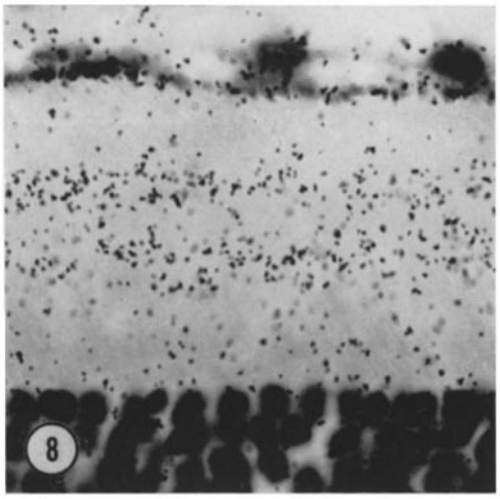
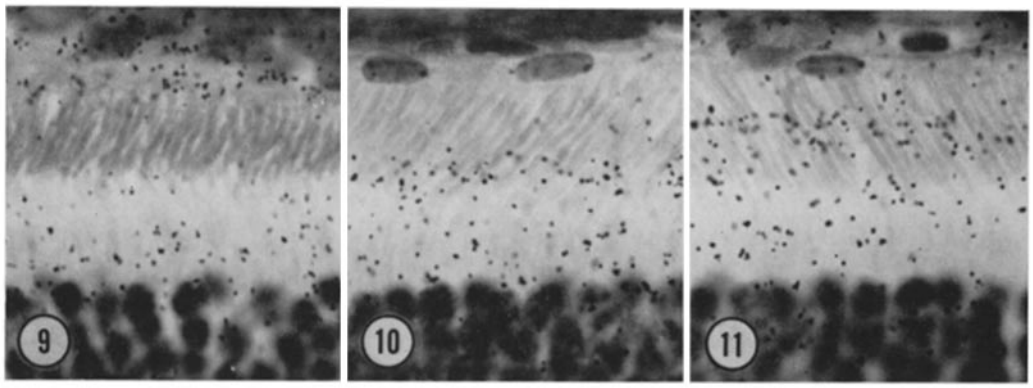


FIGURE 8 Photoreceptor cells from an 8 wk old rat given two injections of methionine-^{3}H, 6 and 2 days before sacrifice. Two reaction bands are visible. This is a characteristic of lamellar apposition. The pigment epithelium (weakly labeled) is visible at the top of the field. Radioautograph; freeze-substitution; hematoxylin. × 1,150.

in intensity during its migration. The outer segment region in advance of the moving band remained unlabeled, whereas a weak reaction occurred immediately behind it. In both types of rods, displacement of the reaction band occurred much more slowly than in rats or mice. In addition, the displacement rate in green rods was slightly less than that in red rods (Figs. 17, 18).[2] The band reached the end of the outer segment (and disappeared) between 6 and 7 wk after injection in red rods, and between 5 and 6 wk after injection in green rods.

Because of the small size of frog cone outer segments, it was not possible to resolve a reaction band with the techniques used. However, radioactivity first was detected in the cone myoids, and subsequently in the outer segments.

[2] In this freeze-substituted material, in which the length of the outer segments of red and green rods averages 32.6 and 21.1 μ, respectively, the rate of band displacement is about 0.74 μ/day in red rods, and 0.57 μ/day in green rods.



FIGURES 9–11 Radioautographs of retinal photoreceptor cells from Swiss albino mice, 8 wk old at the time of injection with methionine-^{3}H. The retinal pigment epithelium and a portion of the choroid are visible at the top of the field. The photoreceptor nuclei are at the bottom. Freeze-substitution; PAS-hematoxylin. × 1,150.

FIGURE 9 1 hr after injection. Radioactivity is concentrated in the inner segments, where it is heaviest in the myoid zone.

FIGURE 10 1 day after injection. Labeled material has been displaced from the inner segments, and is accumulating as a reaction band at the base of the outer segments.

FIGURE 11 5 days after injection. The reaction band has been displaced apically, and occurs near the midpoint of the rod outer segments at this interval.

Effect of Temperature

Differences in ambient temperature had a pronounced and similar effect on the rate of band displacement in both types of rods in the frog during the 10 day experimental period. At 4°C, the process essentially was stopped; a reaction band was still in the process of formation at the base of the outer segments. At 13°C a band had been formed and displaced a short distance. Migration of the reaction band was augmented further at 25°C, and was appreciably greater at 34°C, at which temperature the band was nearly half-way along the outer segment after 10 days (Fig. 21). The displacement rate approximately was doubled with each 10°C increase in ambient temperature.

Effect of Illumination

In both rats and frogs, the displacement of the reaction band at 25°C was increased under conditions of continued, high intensity illumination, and decreased in total darkness (compared to animals maintained at 25°C under standard illumination for comparable durations). The differences were relatively small, but were consistent and reproducible (Fig. 22).

DISCUSSION

The radioautographic reaction observed over tissue sections prepared after administration of labeled amino acids is considered to be due mainly to incorporation in protein (9). Previous reports, which have localized the major part of photoreceptor protein synthesis in the inner segment, have been confirmed in rats, mice, and frogs. Most of the labeled material subsequently was displaced from the inner segment to the outer segment in all three species, as first described in rodents by Droz (8). In the present study, with improved methods of tissue preservation, the radioactivity was observed to move along the outer segment as a discrete reaction band, which did not increase in width or increase in intensity as it proceeded from the base to the apex of the outer segment. This finding has been interpreted to indicate that there is a continual renewal of the entire photoreceptor outer segment (27, 28).

The outer segment is an exceptionally dense mass (23) of hundreds of transversely arranged, lipoprotein discs. The constituent molecules of this lamellar structure are oriented precisely in three dimensions, in what amounts to a "membranous crystal" (5, 11, 25). Each disc consists of a double thickness of infolded, three-layered plasma membrane, in which fusion of the outer membrane layers has produced a compact, five-layered unit, about 150 A thick, separated by interdisc spaces of about 100 A (18) or less (11). Within each disc, the lipids are longitudinally arranged films, and the protein molecules are aligned transversely with respect to the outer segment (21). The visual pigment molecules, as well as the intermediate and final products of their bleaching, also are oriented within this highly differentiated microstructure (4, 26). A renewal mechanism based on a diffusive transport of macromolecules through a medium of this nature appears most unlikely. In fact, the opinion that "no proteins can move in such a structure" has been expressed (25).

The gradual displacement of a discrete, radioactive band is a characteristic of lamellar apposition, as is the appearance of two reaction bands following administration of two injections of labeled amino acid (30). The region in front of the moving band remained unreactive, because no labeled material diffused into it. The region immediately behind the band was labeled weakly. This is believed to be due to the formation of additional layers of outer segment material during the postinjection interval when radioactive precursors continued to be available, but at increasingly lower levels. The concentration of radioactivity in the band itself was stable.[3] Available evidence, then, does not support the interpretation that the labeled protein "flows" or "diffuses" through the outer segment. Instead, it is consistent with the interpretation that the labeled protein is displaced sclerally as a constituent of a stable unit. It appears likely, therefore, that outer segment renewal in mature photoreceptor cells is achieved by the continued lamellar apposition of newly formed discs, which gradually are displaced sclerally by the subsequent addition of newer discs at the base of the outer segments.

This renewal process is apparently in progress during formation of the outer segments, as judged

[3] When the concentration of radioactivity in the band is assessed by grain-count analysis, and compared with the concentration in any other region of the same retina, this concentration ratio actually *increases* with time, as a reflection of the gradual *loss* of radioactivity from the retinal region used for comparison.

by its presence in 4-wk-old (70–85 g) rats, and in the 50 g rats studied by Droz (8).

Outer segment morphogenesis involves the development of a primitive cilium projecting from the inner segment primordium, followed by saclike invaginations of the cell membrane, which flatten and fuse to form five-layered discs. New invaginations are formed repeatedly at the base of the outer segment by successive infolding of the plasma membrane (5, 16, 17, 24). In this way, disc after disc is added, increasing the length of the segment, while displacing the first formed discs in a scleral direction (17). Outer segment renewal in mature cells appears to involve a continuation of this process of disc formation. Retention of the capacity for disc formation in the mature photoreceptor is revealed by the regeneration of damaged rod outer segments during recovery from vitamin A deficiency (6).

Continued addition of membranous discs at the base of the fully developed outer segments implies a balanced removal of material elsewhere. The present findings indicate that this removal occurs at the apical end of the outer segments, which are in contact with the retinal pigment epithelium. In the human retina, it appears that groups of discs

---

FIGURES 12–20 Retinal photoreceptor cells in the frog. The heavily pigmented retinal pigment epithelium is at the top; the photoreceptor nuclei are at the bottom (shown most clearly in Fig. 12). The dark, round or oval bodies seen in the myoid zone of Figs. 13, 15, and 18–20 are portions of cone paraboloids (glycogen deposits). The cone outer segments are not in the plane of section. Figs. 13–20 are radioautographs from adult animals injected with methionine-^{3}H. Freeze-substitution; PAS-hematoxylin. × 1,150.

FIGURE 12 Red and green rods as seen in histological section. Note the relatively short outer segment of the green rod, the base of which is located at a higher level than those of the red rods (see Fig. 1).

FIGURE 13 1 hr after injection of methionine-^{3}H. The radioactivity is concentrated in the myoid portion of the inner segment of red rods.

FIGURE 14 1 hr after injection. The radioactivity also is concentrated in the long, slender myoid portion of the inner segment of green rods. The arrow indicates the base of the outer segment in a green rod.

FIGURE 15 1 day after injection. Most of the labeled material has been displaced to the base of the outer segment, where it has accumulated as a reaction band. The arrow indicates the base of a green rod outer segment.

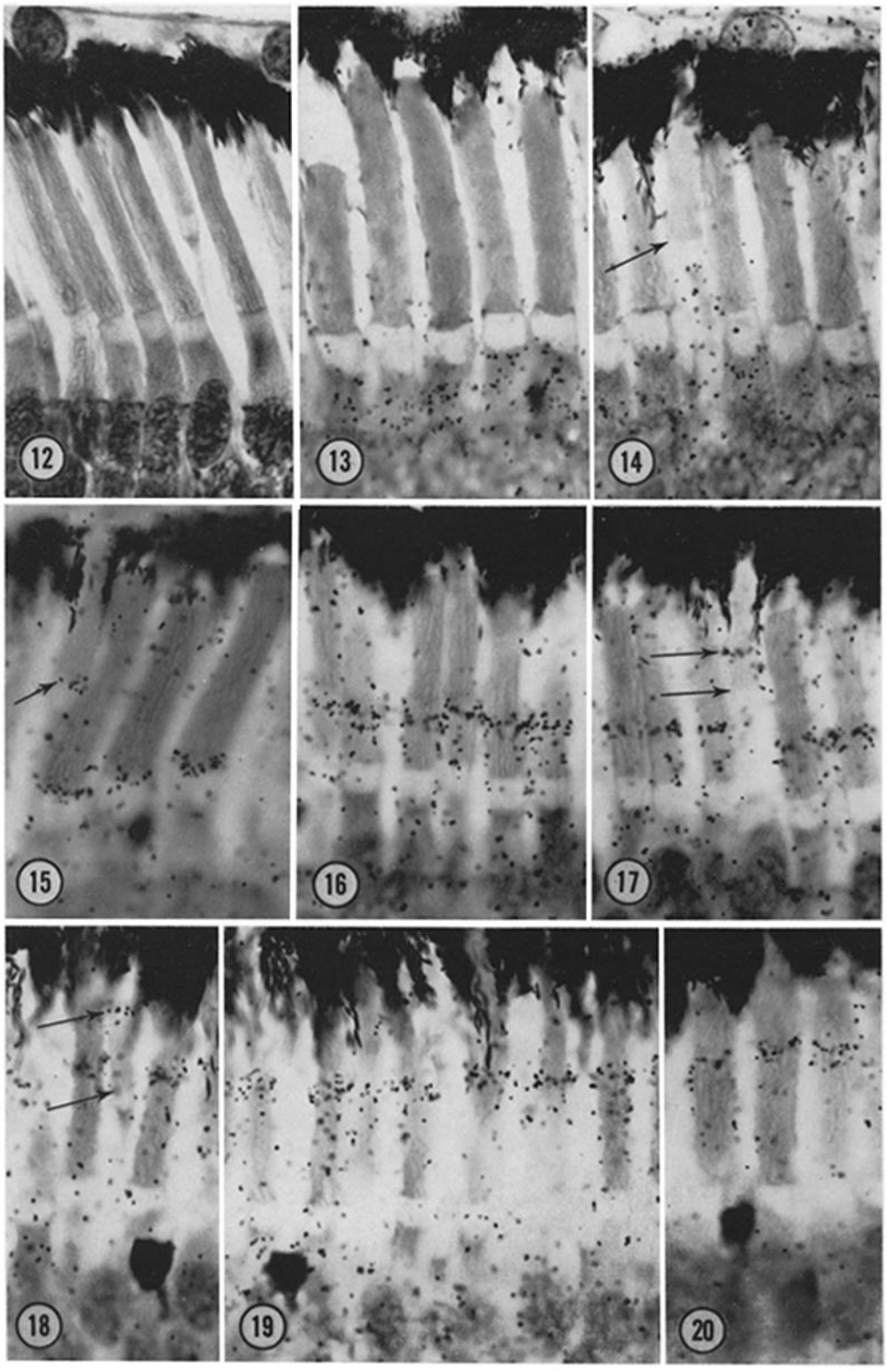
FIGURE 16 1 wk after injection. The reaction band has been displaced sclerally in red rods. The rate of displacement is similar among cells of the same type.

FIGURE 17 1 wk after injection. The reaction band also has been displaced sclerally in green rods. The top arrow indicates the reaction band, the lower arrow the base of the outer segment in a green rod.

FIGURE 18 19 days after injection. The reaction band has been displaced further, towards the apical end of the photoreceptor cells. The arrows indicate the band and the base of the outer segment in a green rod.

FIGURE 19 3 wks after injection. The reaction band is situated at essentially the same level in each of the red rods.

FIGURE 20 24 days after injection. The reaction band is still discrete, and has continued its gradual displacement from the base to the apex of the photoreceptor cell outer segments.


12
13
14
15
16
17
18
19
20

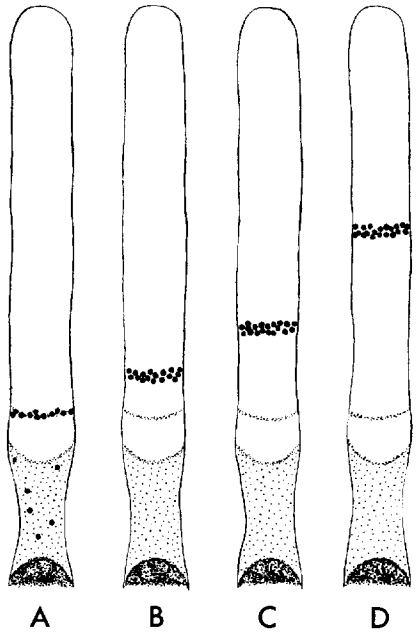


FIGURE 21 Diagrammatic summary of the effects of temperature on the position of the radioactive band in red rods of the frog retina 10 days after injection of methionine-^{3}H. During the experimental period, the frogs were maintained at 4°C (A), 13°C (B), 25°C (C), and 34°C (D).

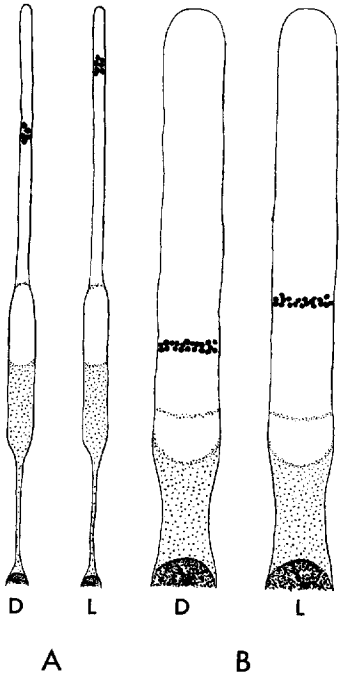


FIGURE 22 Diagrammatic summary of the effects of illumination on the position of the radioactive band in retinal rods of the rat (A) and frog (B), 7 and 10 days after injection, respectively. Half the animals were maintained in complete darkness (D); the remainder were raised under conditions of continual bright light (L).

may be detached from the apex of the outer segment, phagocytized, and absorbed by the pigment epithelium (1). The presence of lysosomes, residual bodies, acid phosphatase, lamellated inclusions, and other indicators of intracellular digestion in pigment epithelial cells of a variety of species (3, 7, 10, 15, 22) supports the concept that this cell type may participate actively in the disposal phase of photoreceptor outer segment renewal.[4]

In frogs, the rate of reaction band displacement was increased when the ambient temperature was raised; this suggests that temperature may be an important rate-controlling factor in the renewal of outer segments in cold-blooded vertebrates. In both frogs and rats, the displacement rate also was accelerated by raising the intensity of retinal illumination, although the effects were not striking, considering that the experimental conditions exceeded normal environmental variation. Perhaps the absorption of light quanta by the visual pigment exerts an influence on lipoprotein synthetic mechanisms, thereby affecting the rate of outer segment renewal (29). Temperature variations in the photoreceptor microenvironment, resulting from absorption of light energy in the retinal pigment epithelium (12), conceivably might participate in such a feedback system.

The results of the present research are interpreted to indicate that the photoreceptor cell outer segment is renewed continually. The renewal mechanism apparently involves the repeated formation of lamellar structures, probably membranous discs, at the base of the outer segment, in conjunction with a balanced removal of material at its apex. The protein components of the outer segment material are synthesized in the myoid

[4] Under such circumstances, it might be possible to detect the presence of labeled outer segment protein in the pigment epithelium 9–11 days after injection of methionine-^{3}H in rats. Further studies are required in this regard, as the current material is equivocal on this point (Fig. 7).

portion of the inner segment. From here they are displaced through a region of concentrated mitochondria, and presumably through the connecting structure in order to attain the base of the outer segment.

This work was supported by United States Public Health Service Grant NB-03807. The valuable technical assistance of Mrs. Mirdza Berzins is acknowledged gratefully.

Received for publication 17 August 1966.

REFERENCES

1. BAIRATI, A., JR., and N. ORZALESI. 1963. The ultrastructure of the pigment epithelium and of the photoreceptor-pigment epithelium junction in the human retina. *J. Ultrastruct. Res.* **9**:484.
2. BOK, D. 1966. RNA and DNA metabolism in rat photoreceptors. *Anat. Record.* **154**:320 (abst.).
3. COHEN, A. I. 1963. Vertebrate retinal cells and their organization. *Biol. Rev.* **38**:247.
4. DENTON, E. J. 1959. The contribution of the oriented photosensitive and other molecules to the absorption of whole retina. *Proc. Roy. Soc. (London) Ser. B.* **150**:78.
5. DE ROBERTIS, E., and A. LASANSKY. 1961. Ultrastructure and chemical organization of photoreceptors. *In* The Structure of the Eye. G. K. Smelser, editor. Academic Press Inc., New York. 29.
6. DOWLING, J. E., and I. R. GIBBONS. 1961. The effect of vitamin A deficiency on the fine structure of the retina. *In* The Structure of the Eye. G. K. Smelser, editor. Academic Press Inc., New York. 85.
7. DOWLING, J. E., and I. R. GIBBONS. 1962. The fine structure of the pigment epithelium in the albino rat. *J. Cell Biol.* **14**:459.
8. DROZ, B. 1963. Dynamic condition of proteins in the visual cells of rats and mice as shown by radioautography with labeled amino acids. *Anat. Record* **145**:157.
9. DROZ, B., and H. WARSHAWSKY. 1963. Reliability of the radioautographic technique for the detection of newly synthesized protein. *J. Histochem. Cytochem.* **11**:426.
10. FEENEY, L., J. GRIESHABER, and J. ALVARADO. 1966. New observations on human retinal pigment epithelium. *Invest. Ophthalmol.* **5**:111 (abst.).
11. FERNANDEZ-MORAN, H. 1962. Cell membrane ultrastructure: Low temperature electron microscopy and x-ray diffraction studies of lipoprotein components in lamellar systems. *In* Ultrastructure and Metabolism of the Nervous System. S. R. Korey, A. Pope, and E. Robins, editors. Williams and Wilkins, Baltimore. 235.
12. GEERAETS, W. J., R. C. WILLIAMS, G. CHAN, W. T. HAM, JR., D. GUERRY, and F. H. SCHMIDT. 1962. The relative absorption of thermal energy in retina and choroid. *Invest. Ophthal.* **1**:340.
13. HALL, M. O., D. E. OCUMPAUGH, and R. W. YOUNG. 1965. The utilization of ^{35}S-sulfate in the synthesis of mucopolysaccharides by the retina. *Invest. Ophthal.* **4**:322.
14. MARAINI, G., R. FRANGUELLI, and S. PERALTA. 1963. Studies on the metabolism of the retina and lateral geniculate nucleus. *Invest. Ophthalmol.* **2**:567.
15. MISSOTTEN, L. 1964. L'ultrastructure des tissus oculaires. *Bull. Soc. Belge Ophtalmol.* **136**:1.
16. MOODY, M. R., and J. D. ROBERTSON. 1960. The fine structure of some retinal photoreceptors. *J. Biophys. Biochem. Cytol.* **7**:87.
17. NILSSON, S. E. G. 1964. Receptor cell outer segment development and ultrastructure of the disk membranes in the retina of the tadpole (*Rana pipiens*). *J. Ultrastruct. Res.* **11**:581.
18. NILSSON, S. E. G. 1965. Ultrastructure of the receptor outer segments in the retina of the leopard frog (*Rana pipiens*). *J. Ultrastruct. Res.* **12**:207.
19. NOVER, A., and B. SCHULTZE. 1960. Autoradiographische Untersuchung über den Eiweissstoffwechsel in den Geweben und Zellen des Auges. *Arch. Ophthalmol.* **161**:554.
20. OCUMPAUGH, D. E., and R. W. YOUNG. 1966. Distribution and synthesis of sulfated mucopolysaccharides in the retina of the rat. *Invest. Ophthalmol.* **5**:196.
21. SCHMIDT, W. J. 1961. Polarisationsoptische Analyse der Verknüpfung von Protein- und Lipoidmolekeln, erläutert am Aussenglied der Sehzellen der Wirbeltiere. *Pubbl. Staz. Zool. Napoli.* **23** (Suppl.): 158.
22. SHANTHAVEERAPPA, T. R., and G. H. BOURNE. 1964. Histochemical studies on the distribution of acid phosphatase in the eye. *Acta Histochem.* **18**:317.
23. SIDMAN, R. L. 1957. The structure and concentration of solids in photoreceptor cells studied by refractometry and interference microscopy. *J. Biophys. Biochem. Cytol.* **3**:15.

24. SJÖSTRAND, F. S. 1961. Electron microscopy of the retina. *In* The Structure of the Eye. G. K. Smelser, editor. Academic Press Inc., New York. 1.
25. WALD, G. 1961. General discussion of retinal structure in relation to the visual process. *In* The Structure of the Eye. G. K. Smelser, editor. Academic Press Inc., New York. 101.
26. WALD, G., P. K. BROWN, and I. R. GIBBONS. 1963. The problem of visual excitation. *J. Opt. Soc. Am.* **53**:20.
27. YOUNG, R. W. 1965. Renewal of photoreceptor outer segments. *Anat. Record.* **151**:484 (abst.).
28. YOUNG, R. W. 1966. Further studies on the renewal of photoreceptor outer segments. *Anat. Record.* **154**:446 (abst.).
29. YOUNG, R. W. 1967. The organization of vertebrate photoreceptor cells. *In* The Retina: Morphology, Function and Clinical Characteristics. R. Allen, M. Hall, and B. Straatsma, editors. University of California Press, Los Angeles. In press.
30. YOUNG, R. W., and R. C. GREULICH. 1963. Distinctive autoradiographic patterns of glycine incorporation in rat enamel and dentine matrices. *Arch. Oral Biol.* **8**:509.