

THE RENEWAL OF ROD AND CONE OUTER SEGMENTS IN THE RHESUS MONKEY

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ABSTRACT

The renewal of retinal rod and cone outer segments has been studied by radioautography in rhesus monkeys examined 2 and 4 days after injection of leucine-³H. The cell outer segment consists of a stack of photosensitive, membranous discs. In both rods and cones some of the newly formed (radioactive) protein became distributed throughout the outer segment. Furthermore, in rods (but not in cones), there was a transverse band of concentrated radioactive protein slightly above the outer segment base 2 days after injection. This was due to the formation of new discs, into which labeled protein had been incorporated. At 4 days, these radioactive discs were located farther from the outer segment base. Repeated assembly of new discs had displaced them away from the basal assembly site and along the outer segment. Measurements of the displacement rate indicated that each retinal rod produces 80–90 discs per day, and that the entire complement of outer segment discs is replaced every 9–13 days. To compensate for the continual formation of new discs, groups of old discs are intermittently shed from the apical end of the cell and phagocytized by the pigment epithelium. Each pigment epithelial cell engulfs and destroys about 2000–4000 rod outer segment discs daily. The similarity between visual cells in the rhesus monkey and those in man suggests that the same renewal processes occur in the human retina.

INTRODUCTION

The detection of light by photoreceptor cells is the fundamental basis for the process of vision. In the vertebrate eye, light is detected when its energy is absorbed by pigment molecules located in the outer segments of retinal rods and cones. The cell outer segment consists of a remarkable stack of hundreds of disc-shaped membranes, piled one upon the other, and enclosed within the cell membrane (Fig. 1). This compact, repetitive arrangement serves to concentrate, stabilize, and orient the photosensitive molecules, which are structural components of the discs.

In the rat, mouse, and frog, the outer segments of rod visual cells undergo continual renewal (24). This was discovered by injecting animals with radioactive amino acids, then using radioautog-

raphy to trace the fate of the labeled proteins which the visual cells synthesize from the labeled precursors. Radioactive protein first appears in the region of high ribosome content in the cell inner segment, the site of protein synthesis (9, 24, 32). Protein destined for the outer segment flows through the Golgi complex, then traverses the connecting cilium to reach the base of the outer segment, where most of it is used in the assembly of new discs (25, 32). Much of this protein is opsin, the protein component of rod visual pigment (2, 12, 13, 16).

Repeated assembly of new membranous discs at the base of the rod outer segment displaces the radioactive discs away from the base and toward the apical end of the cell, which is inserted in the

pigment epithelium (Fig. 1). When this process is observed by radioautography, the labeled discs appear as a transversely oriented band of radioactivity which moves gradually along the outer segment. Upon reaching the end of the cell, the radioactive discs are detached from it, and subsequently can be identified in inclusion bodies (phagosomes) in the cytoplasm of the pigment epithelial cells (30, 31). Thus, the pigment epithelium scavenges the old discs which are shed from the tip of the rod visual cell to compensate for the continual formation of new discs at the outer segment base. In this way, the length of the segment remains essentially constant in the mature cell.

The two classes of visual cells, rods and cones, are distinguished by differences in the shape of the outer segment. In rods the outer segment is cylindrical. In cones it is conical or tapered; that is, the discs at the top of the stack are smaller than those at the base. Available evidence suggests that the process of outer segment renewal differs in these two types of photoreceptor cells.

Cone visual cells do not continually produce new outer segment discs, at least in the mature retina of the frog and salamander, the only animals with retinal cones to be studied so far (26, 28). Radioautography shows that new (labeled) protein is continually transferred from the inner to the outer segment in cones as well as in rods. However, in cones it scatters within the outer segment rather than becoming concentrated in newly forming discs.

Do similar renewal processes occur in the human eye? The question remains unanswered. However, if it can be shown that comparable renewal mechanisms are invariably operative in other vertebrate species, it then can be assumed with some confidence that they occur also in the visual cells of the human retina, for these cells are in no way unique in their organization (7, 27). An analysis of rod and cone outer segment renewal in the rhesus monkey is of particular interest, because the visual cells in this animal are remarkably like those of the human in their size, shape, retinal distribution, and physiology. The results of such an analysis are presented in the report which follows.

MATERIALS AND METHODS

Two rhesus monkeys (*Macaca mulatta*) were used. One was female, 12 months of age, and weighed 1850 g; the other was male, 8 months of age, and

weighed 1740 g. The monkeys, tranquilized with Sernylin (Parke, Davis & Co., Detroit, Mich.), were injected in the saphenous vein with 70 mCi of leucine-4,5-³H (Schwarz Bio Research Inc., Orangeburg, N.Y., 58 Ci/mmmole) dissolved in 1 ml of 0.9% NaCl. The animals were killed 2 and 4 days after injection, respectively.

At the conclusion of the experiment, the monkeys were placed under deep anesthesia by intravenous injection of Nembutal (Abbott Laboratories, Chicago, Ill.), then killed by intracardiac vascular perfusion of an aldehyde fixative at a pressure of 90 mm Hg. The first animal (2 days postinjection) was perfused with a solution of 0.8% glutaraldehyde in pH 7.6 phosphate buffer (22). The second animal was perfused with a solution of 1% formaldehyde and 1% glutaraldehyde in pH 7.1 phosphate buffer (3.41 g NaH₂PO₄·H₂O and 21.84 g Na₂HPO₄·12 H₂O per liter distilled water).¹

The posterior two-thirds of the ocular globe was fixed overnight in the aldehyde solution at 4°C. A square block of tissue, about 2.5 mm × 2.5 mm with the fovea at its center, was then removed from each eye. The zone immediately surrounding the fovea, and included in this block, is termed the parafovea. (The diameter of the fovea + parafovea is about 2.5 mm; reference 19). Blocks from the immediately surrounding region, the perifovea, were also separated. (The central area of the retina, consisting of the fovea, parafovea, and perifovea, is 5–6 mm in diameter; reference 19). Other specimens were taken from the periphery of the retina, including some from the far periphery, near the ora serrata. Specimens from these several zones were then fixed for 1 hr in 1% osmium tetroxide in the appropriate phosphate buffer and subsequently were embedded in Araldite.

Radioautograms were prepared as follows: Tissue specimens were reoriented so that longitudinal sections of the visual cells could be obtained. Sections were cut at a thickness of 0.5 μ, and collected on glass microscope slides. After removal of the plastic embedment by incubation in an ethanolic solution of NaOH, the sections were dipped in Kodak NTB2 emulsion which had been diluted 1:1 with distilled

¹ Although fixation with glutaraldehyde washes out much of the low molecular weight material from tissues, it can result in the binding of some free amino acids to tissue proteins. Therefore, glutaraldehyde must be avoided in protein studies which are terminated within minutes after administration of labeled amino acids, when the level of radioactive free amino acid is still high (18). In long-term studies of the type herein described, this potential source of artifact may be considered insignificant. The residual tissue reaction is due almost entirely to newly synthesized protein (3).

water and was maintained at 40°C. The preparations were exposed 6–8 wk at 4°C, then developed in Kodak Dektol for 2 min at 17°C. Next, they were fixed in Kodak Acid Fixer, washed in water, and stained with 1% toluidine blue in 1% sodium borate.

Additional specimens were reoriented so that transverse sections could be obtained. These were examined by light microscopy after staining with toluidine blue. Ultrathin sections (transverse and longitudinal), prepared for examination in the electron microscope, were stained with uranyl acetate and lead citrate.

The length of the rod outer segment and the distance between the base of the outer segment and the transverse band of radioactivity (Fig. 1) were determined in radioautograms by means of an ocular micrometer. These dimensions were measured on 100 rods in the parafoveal, perifoveal, and peripheral regions of the retina in each animal. Renewal time for the rod outer segment was estimated by relating the distance of disc displacement to total outer segment length, using the relationship shown in Fig. 1.

The average number of discs per rod outer segment was counted in rods located in the perifoveal and peripheral regions. Longitudinal sections which passed through the full length of the outer segment were photographed in the electron microscope and printed at a final enlargement of about 3000, then analyzed with the dissecting microscope. Such analysis requires perfect cellular orientation, which was not obtained in the parafovea. Consequently, the number of discs per rod outer segment in this region was calculated indirectly from the relative length of the outer segments, compared to that in the two regions where the discs were counted directly.

In transverse sections prepared for light microscopy, the distribution of rods, cones and pigment epithelial cells in the fovea, parafovea, perifovea, periphery, and far periphery of the retina was as-

essed as follows: Photographs of the pigment epithelium and visual cell outer segments were printed at a final enlargement of 1800. In each region, 25 pigment epithelial cells were cut out of the photographs and weighed. The average area occupied by the cells was then obtained from a standard curve relating paper weight to area. This area was

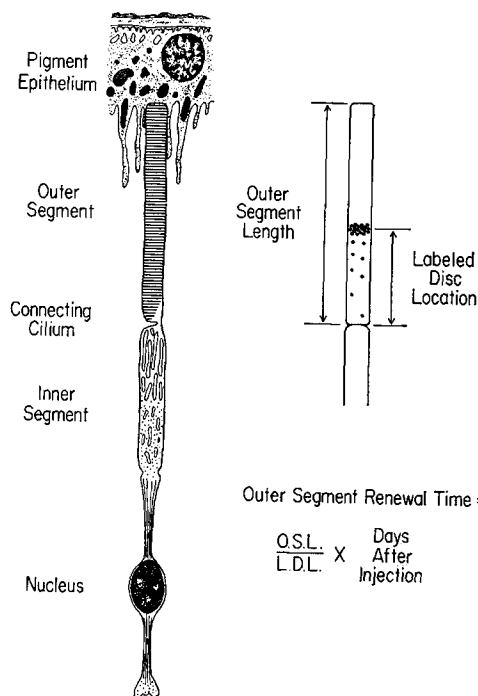


FIGURE 1. Diagram illustrating the major components of a vertebrate visual cell. Note that the apical end of the outer segment is embedded in the cytoplasm of the pigment epithelium. The method for estimating outer segment renewal time is presented on the right.

TABLE I
Location of Labeled Discs, Length of Rod Outer Segments, and Estimated Rod Outer Segment Renewal Time in Different Regions of the Retina, 2 and 4 Days after Injection of Leucine-³H

	Parafovea		Perifovea		Periphery	
	2 days	4 days	2 days	4 days	2 days	4 days
Labeled disc location (in μ)*	5.6	10.8	5.5	10.4	5.5	10.6
Outer segment length (in μ)	36.5	33.9	32.2	30.2	25.3	22.5
Outer segment renewal time (in days) ‡	13.0	12.6	11.7	11.6	9.1	8.5

* Distance from the base of the outer segment to the level of the heavily labeled discs (Fig. 1).

‡ $\frac{\text{Outer segment length}}{\text{Labeled disc location}} \times \text{days after injection.}$

then corrected for photographic enlargement. The number of rods and cones per unit area was measured by superimposing a square of known area over the photographs depicting visual cell outer segments in transverse section, and by counting the total number of cells of each type within that area.² 25 such areas

² Cone outer segments can be distinguished in transverse sections examined under low magnification because they are surrounded by a prominent space filled with extracellular material and processes of the pigment epithelium. This space results from the fact that cone inner segments in the rhesus monkey (as in the human) are much bulkier than those of the rods, whereas the outer segments are of roughly comparable diameter. In the rod-free fovea, the cone inner segments are very slender, and the surrounding space is absent.

(comprising $700.0\mu^2$ each) were analyzed in each region.

Since the cross-sectional area of the pigment epithelial cells had been measured, and since the number of rods and cones per unit area was also known, the number of rods and cones associated with each pigment epithelial cell could then be calculated.

RESULTS

Two days after injection of leucine-³H, a distinct transverse band of radioactivity was detected by radioautography about 5.5μ above the base of each rod outer segment (Table I; Figs. 2-7). At 4 days, the radioactive band had moved. It now was located about 11μ from the outer segment base (Table I; Fig. 8), but had not increased in

FIGURES 2-8 Radioautograms depicting rods and cones, 2 and 4 days after injection of leucine-³H. The pigment epithelium is visible at the top of each photograph. 0.5μ sections, stained with toluidine blue, are shown. $\times 1060$.

FIGURE 2 2 days after injection of leucine-³H, a transverse band of radioactive protein (upper arrow) is present near the base (lower arrow) of each rod outer segment. In contrast, no such concentration of radioactivity appears in the outer segment of the cone (c, far left). *pe*, pigment epithelium; *is*, rod inner segments.

FIGURE 3 The outer segments of two cones are visible (arrows). Both contain newly formed, radioactive protein, which is not, however, localized as in the rods, 2 days after injection. The scattered, relatively weak radioautographic reaction is comparable to that present in the outer part of the rod outer segments. In both instances newly formed protein has penetrated the pre-existing disc structure.

FIGURE 4 The zone of concentrated labeled protein near the base of each rod is considered to be due to a group of intensely labeled discs formed immediately after the injection, 2 days earlier, and now displaced partway along the outer segment as a result of continued disc formation. There is no evidence of new disc assembly in the cone (arrow).

FIGURE 5 The radioactive discs have been displaced a similar distance from the base in each rod, indicating comparable rates of disc production. The three cone outer segments (arrows) contain relatively small amounts of diffusely distributed new protein.

FIGURE 6 The radioautograms reveal that the process of outer segment renewal differs in rods and cones. Labeling is weak and diffuse in the cone outer segment (arrow) and in the outer part of the rod outer segments (*x*). The discs near the base of the rod outer segments are heavily labeled because they were assembled during the 2 days since the injection of radioactive leucine.

FIGURE 7 2 days after injection, newly formed, intensely radioactive discs (middle arrow) have been displaced a short distance from the base (lower arrow) of the rod outer segment. By measuring that distance, and the length of the rod outer segment (distance between upper and lower arrows), it is possible to estimate the rod outer segment renewal time (cf. Fig. 1). This proved to range between 9 and 13 days in different parts of the retina.

FIGURE 8 4 days after injection, the intensely radioactive discs (middle arrow) have been further displaced. They are now situated approximately halfway between the base (lower arrow) and the extremity (upper arrow) of the outer segment. The rods in Fig. 7 are from the parafovea; those in Fig. 8 are from the periphery of the retina. Note that the rod outer segments are shorter in the periphery.

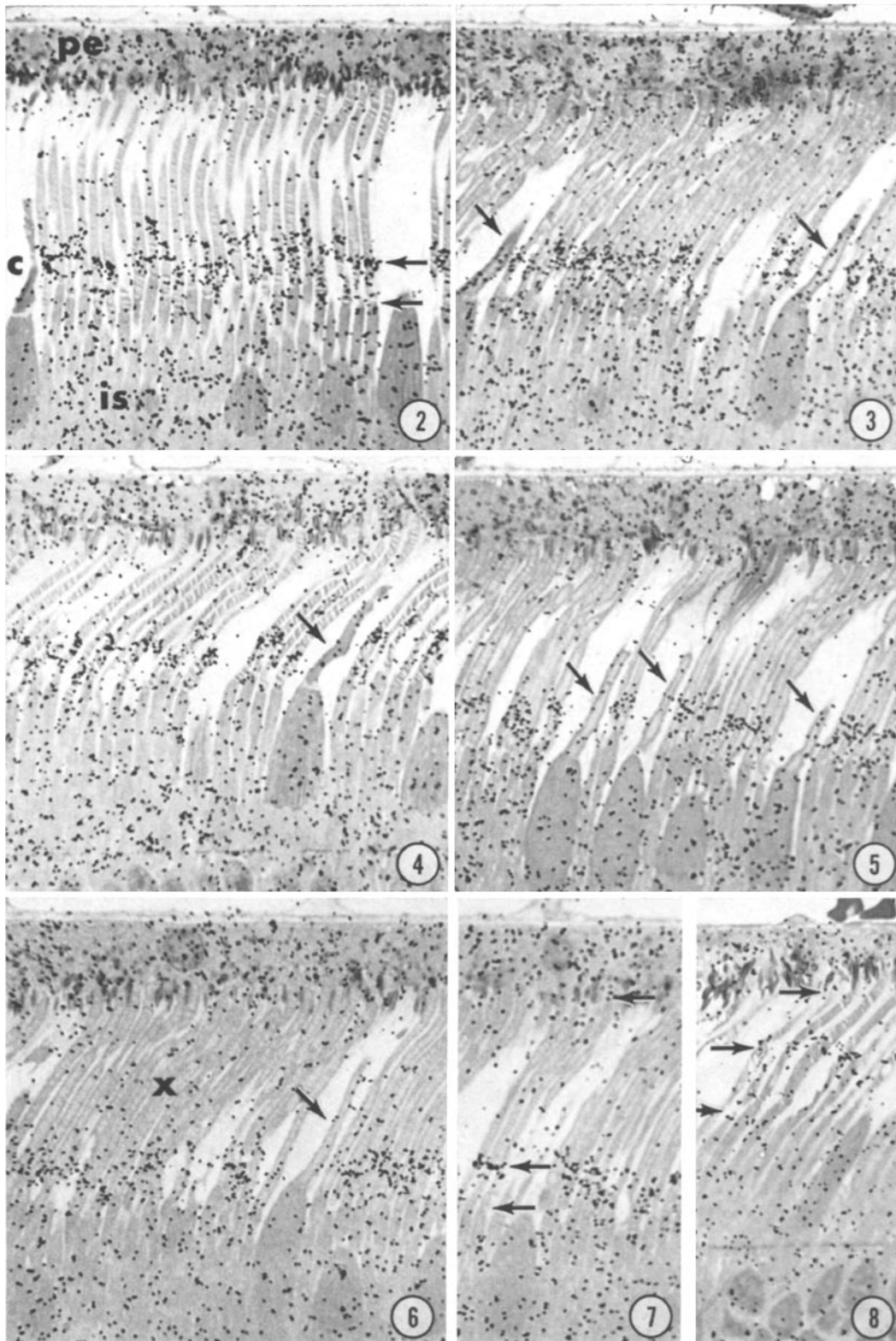


TABLE II
Number of Discs per Rod, Outer Segment Renewal Time, and Rate of Disc Formation in Different Regions of the Retina

	Parafovea	Perifovea	Periphery
Number of discs per rod	1100	920	790
Outer segment renewal time*	12.8 days	11.6 days	8.8 days
Number of discs per day	86.7	79.3	89.8

* Average of two estimates given in Table I.

width or decreased in labeling intensity. In contrast, cone outer segments did not contain such localized concentrations of labeled protein in any area of the retina, including the fovea, at either interval after injection (Figs. 2-6).

Rod outer segments were longest in the parafovea, and gradually decreased in length towards the periphery. Because the location of the labeled discs was similar throughout the retina in each animal, whereas the lengths of the outer segments differed regionally, the estimated outer segment renewal times varied according to the region (Table I). In the parafovea, the estimated renewal time was 12.6-13.0 days; in the perifovea, it was 11.6-11.7 days; and in the periphery, 8.5-9.1 days.

A diffuse scattering of silver grains over cone outer segments attested to the fact that some newly

formed (radioactive) protein had been transported to this part of the cell. In rods, the outer segments were diffusely but weakly labeled beyond the level of the intensely reactive discs (towards the pigment epithelium). Behind the heavily labeled discs (towards the outer segment base), there was a heavier, scattered labeling.

The average number of discs per rod outer segment, counted directly in electron micrographs, was 920 in the perifovea and 790 in the periphery of the retina. In the parafovea, the average number of discs was computed to be about 1100, due to the greater length of the outer segments in this region (Table II).

On the basis of these totals, and an estimated outer segment renewal time for each region averaged from the values obtained in the two experi-

FIGURES 9-16 Transverse sections through the pigment epithelium and visual cell outer segments in different regions of the retina. Note the regional variation in the cross-sectional area of individual pigment epithelial cells, and in the distribution of rods and cones. The sections are 1 μ thick, and stained with toluidine blue. \times 830.

FIGURE 9 Pigment epithelium, fovea. The hexagonal cells have the smallest transverse dimensions in this region.

FIGURE 10 Visual cell outer segments, fovea. Only cones are present. They are very slender and densely packed.

FIGURE 11 Pigment epithelium, parafovea.

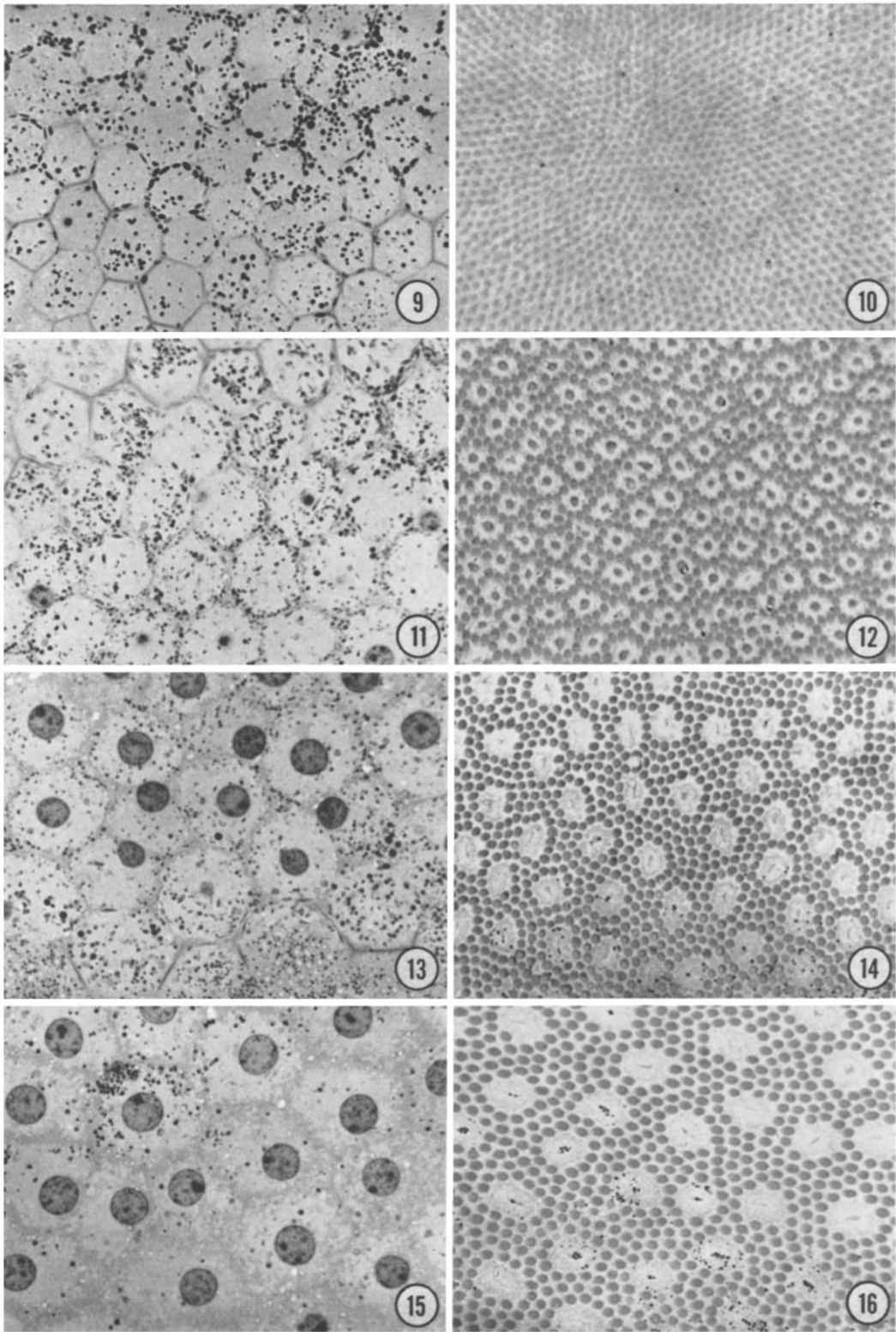
FIGURE 12 Visual cell outer segments, parafovea. Most of the cones are surrounded by a single row of rods.

FIGURE 13 Pigment epithelium, periphery. The section passes through the cell nuclei.

FIGURE 14 Visual cell outer segments, periphery. The section passes above the ends of the cone outer segments, which are represented by a space surrounded by two or three rows of rods.

FIGURE 15 Pigment epithelium, far periphery. The cells are approximately three times larger in cross-sectional area than those in the fovea.

FIGURE 16 Visual cell outer segments, far periphery. The ratio of rods to cones is further increased, the cells are less densely packed, and the rod outer segments are slightly thicker compared to more central regions.



ments (Table I), it was calculated that about 80–90 discs were assembled daily by each rod (Table II).

Rods outnumbered cones in all regions of the retina, except the center of the fovea, where only cones were present. Cell density in the central fovea (in a circular area 80 μ in diameter) was nearly twice that in any other region (Figs. 10, 12, 14, 16). The concentration of cones dropped sharply in the parafovea, then declined gradually in the far periphery (Table III). Rods outnumbered cones 5:1 in the parafovea, and about 17:1 in the periphery. In the far periphery, the ratio of rods to cones was nearly 23:1, and the visual cells were less densely packed.

Rods were arranged in circles around the cones. In the parafovea, each cone was enclosed within a simple circle of rods. In the far periphery, there were three or four circles of rods surrounding each cone (Figs. 12, 16).

The cross-sectional area occupied by individual pigment epithelial cells was smallest in the fovea, and increasingly larger towards the periphery. In the far periphery, each pigment epithelial cell covered an area three times greater, on the average, than that occupied by comparable cells in the fovea (Figs. 9, 11, 13, 15).

From these results, it could be calculated (Table III) that for most of the retina 39–45 rods and two or three cones were associated with each pig-

ment epithelial cell. In the parafovea, there were fewer rods (24–25) and more cones (4–5) related to each cell in the pigment epithelium.

The disc diameter in the cylindrical rod outer segments was 1.5 μ near the fovea, and increased slightly to about 1.7 μ in the far periphery. In cones, discs at the tip of the outer segment were 1.5 μ in diameter; those near the base had a diameter of 3.0 μ in the parafovea and 3.5 μ in the periphery. In the fovea, the discs were 1.8 μ wide at the base of the outer segment, and 1.2 μ at the tip. (This measurement was made slightly off the center of the fovea; cone outer segments at the foveal center were of even smaller diameter.)

The basal discs of both rods and cones were continuous with the cell membrane (Figs. 18–20). At a variable distance above the base, generally 6–10 discs, continuity appeared to be lost in rods. In cones, this process was more irregular, so that attached and unattached discs were intermingled for an appreciable distance above the base (Fig. 20). Towards the apical end of the outer segment, the discs appeared to be unconnected with the cell membrane in cones as well as in rods, although it is possible that connections existed which were missed because the sections did not pass through them.

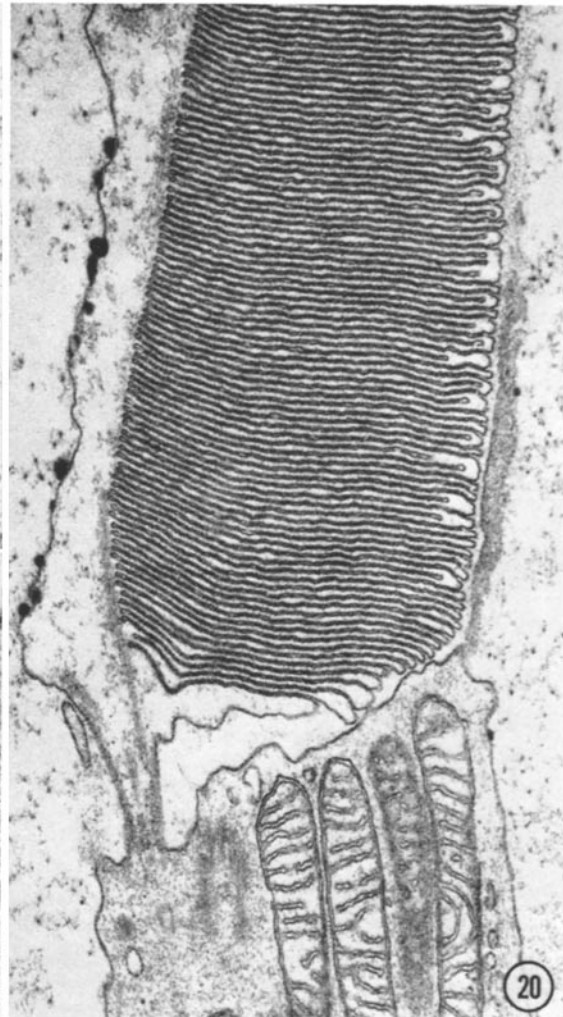
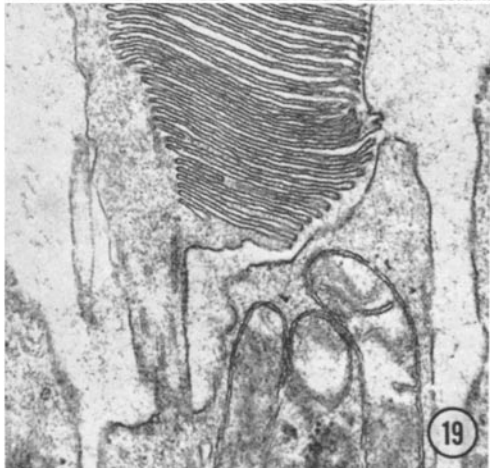
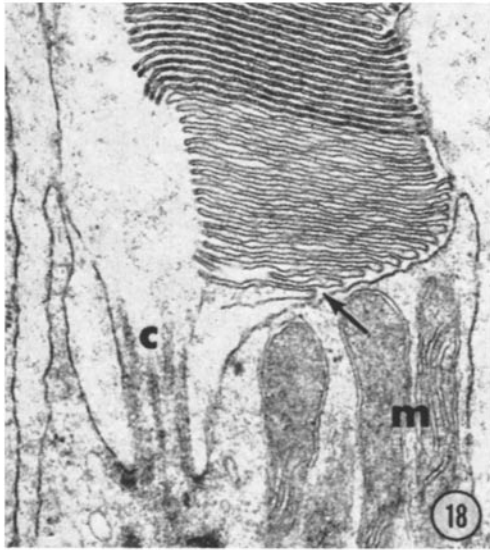
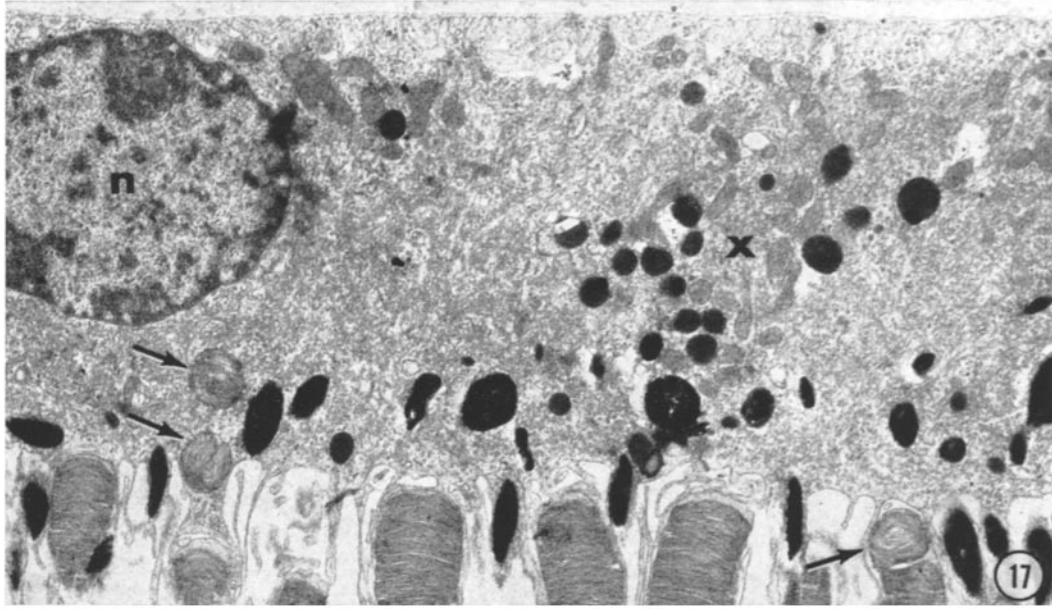
In rods, the discs had a tendency to swell irregularly in the 0.8% glutaraldehyde fixative (Fig. 19). In the other aldehyde fixative, there was

FIGURE 17 Pigment epithelium in the peripheral region of the retina. Cytoplasmic extensions enclose the tips of the rod outer segments (below). Two phagosomes, containing detached rod outer segment discs, are visible at the lower left (arrows). A group of discs is being shed from the end of a rod (arrow, lower right). Dense inclusions and mitochondria tend to accumulate near the lateral margins of the epithelial cells (*x*). Mitochondria also aggregate near the infolded basal surface (above). The cytoplasm is filled with smooth-surfaced endoplasmic reticulum. *n*, nucleus. Electron micrograph. $\times 7700$.

FIGURE 18 Base of a rod outer segment. The youngest discs, nearest the base, are open to the extracellular space (arrow), as if formed by infolding of the cell membrane. Shortly after formation, the discs appear to lose their attachment to the outer membrane. These older discs are more regular and uniform in appearance. This tissue was fixed in 1% formaldehyde, 1% glutaraldehyde. *c*, connecting cilium; *m*, mitochondria in the inner segment. Electron micrograph. $\times 36,200$.

FIGURE 19 Base of a rod outer segment, fixed in a solution of 0.8% glutaraldehyde. The appearance is similar (compared to Fig. 18), except that with this fixative, all of the discs appear slightly swollen. Note that the discs near the base are continuous with the outer cell membrane. Electron micrograph. $\times 31,640$.

FIGURE 20 Base of a foveal cone outer segment. The lowermost discs are continuous with the cell membrane, but at higher levels some of the discs appear to have lost this attachment. There is no apparent swelling of the discs, in contrast to rods preserved in the same 0.8% glutaraldehyde solution (Fig. 19). Electron micrograph. $\times 31,640$.



no obvious swelling of the discs (Fig. 18). In these well-preserved outer segments, there were 38.7 discs per μ in rods and 31.0 discs per μ in cones. Cone discs were round; those of rods were lobulated at the edges (Fig. 21). In perifoveal rods, the average number of shallow indentations was nine (range, 6–12).

Except in the fovea, the cone outer segments were significantly shorter than those of the rods. Cytoplasmic processes of the pigment epithelial cells were draped over the ends of both, extending up to 40% of the distance down the rod outer segments, but just reaching the tips of the cones.

Phagosomes containing groups of detached rod outer segment discs were a prominent feature of the pigment epithelium (Figs. 17, 22–25). Groups of discs in the process of undergoing detachment from the ends of rod outer segments were observed in all parts of the retina (Figs. 17, 22, 23). Aggregations of 10–20 discs frequently were shed at one time. After ingestion by the pigment epithelium, the discs within the phagosomes were displaced deeper into the cytoplasm, often turning and rotating in the process, and becoming more densely compacted in spherical whorls (Figs. 17, 22–25).

DISCUSSION

2 days after the intravenous injection of leucine- ^3H , an amino acid used predominantly in the synthesis of protein, an intense, transversely oriented band of radioactive protein was observed by radioautography about 5.5 μ from the base of each

retinal rod outer segment. 2 days later, the band of labeled material appeared at a level twice that distance from the outer segment base. It had not increased in thickness, nor had it decreased in intensity. This progressive shift along the rod outer segment of newly formed protein concentrated in a stable, disc-shaped unit results from the gradual displacement of membranous discs which were assembled at the base of the outer segment in the hours immediately following the injection (24, 32).

Discs located nearer the base of the outer segment were less intensely labeled. These were the newest discs, assembled after the sudden pulse of radioactivity had passed, and during the period when radioactive precursor molecules were available at a reduced and progressively decreasing level (24).

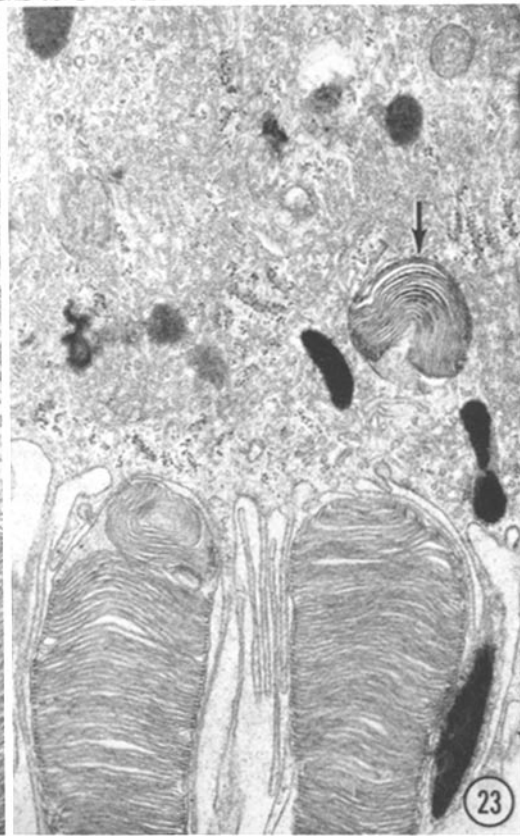
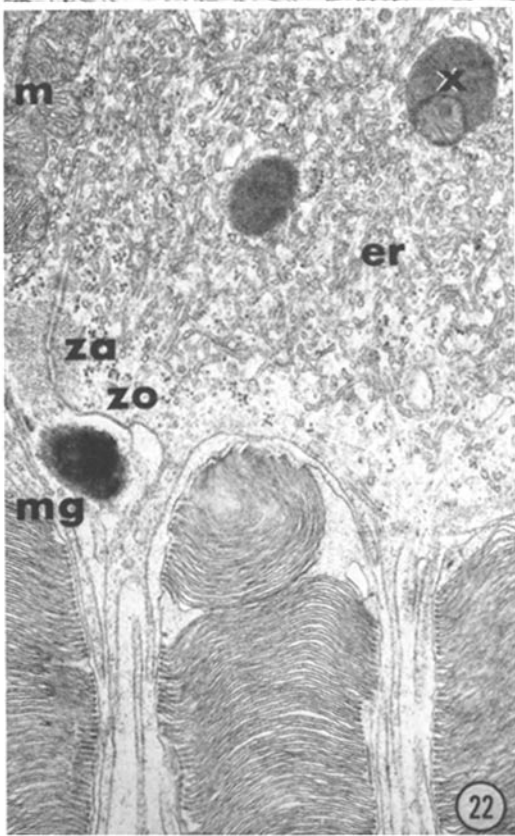
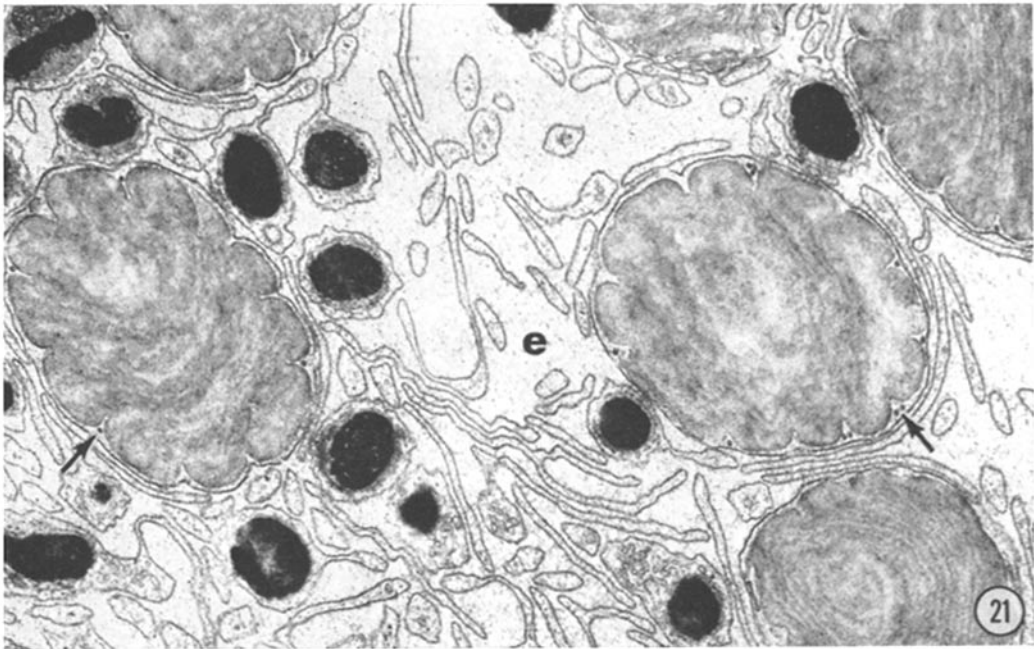
The weaker labeling of the zone of older discs, located on the pigment epithelium side of the heavily reactive ones, is due to a different process. These discs were formed *before* the injection of radioactivity. This part of the outer segment became labeled because a small amount of newly formed protein infiltrated the existing disc structure. Experiments in the frog have shown that the diffusely distributed protein is synthesized in the inner segment of the cell (Bok and Young, unpublished).

There was a remarkable uniformity in the rate of disc formation by rod visual cells. The heavily labeled discs had been displaced the same distance from the rod outer segment base, without signifi-

FIGURE 21 Transverse section through several rod outer segments near the junction with the pigment epithelium. The periphery of the discs is scalloped. Longitudinally oriented microtubules (arrows) are present between the disc perimeter and the outer cell membrane in some of the indentations. The space between the rod outer segments is filled with extracellular material (*e*), believed to consist largely of mucopolysaccharide, and with delicate processes of the pigment epithelium, some of which contain melanin granules. Electron micrograph. $\times 28,000$.

FIGURE 22 Part of the tips of three rod outer segments (below) are seen at their junction with the pigment epithelium. Several discs are undergoing detachment from the end of the rod in the center. *m*, mitochondria; *er*, endoplasmic reticulum (predominantly smooth-surfaced); *mg*, melanin granule; *za*, zonula adherens, and *zo*, zonula occludens between adjacent epithelial cells. The structure in the upper right corner of the field (*x*) may represent a stage in the destruction of phagocytized rod outer segment discs. Electron micrograph. $\times 23,275$.

FIGURE 23 Junction of the rod outer segments with the pigment epithelium. A group of discs is being shed from the end of the cell on the left. A comparable group of discs, previously detached, has been engulfed by the pigment epithelium, and is contained within a phagosome (arrow). Electron micrograph. $\times 17,150$.



cant variation, in all regions of the retina. A similar regularity in the rate of disc production has been observed in rat and mouse rods (24). In the frog, there are two types of retinal rod visual cells. The disc assembly rate differs between the two types, but rods of the same type produce discs at a constant rate throughout the retina (24, 32).

Because disc formation appears to be continuous and steady, it is possible to predict from a single postinjection interval the length of time which would be required for the discs to be displaced the entire length of the outer segment, and thus the time required for renewal of the entire complement of rod outer segment discs. (Thus, for example, if the new, intensely radioactive discs have been displaced one-half the length of the outer segment in 5 days, the outer segment renewal time is 10 days). In this study, two postinjection intervals were available from which the rod outer segment renewal time could be calculated. The results of these independent estimates were remarkably similar.

The estimated rod outer segment renewal time varied regionally, because the length of the rod outer segments progressively decreased towards the periphery of the retina. In the parafovea, the renewal time (averaged from the two estimates) was 12.8 days; in the perifovea, it was 11.6 days; in the periphery, 8.8 days. This is similar to the renewal rate in rats and mice, in which the rod outer segment discs are completely replaced in 9–10 days (24).

The average number of discs contained in each rod outer segment was 1100 in the parafovea, 920 in the perifovea, and 790 in the periphery of the monkey retina. Since the time required to replace the entire complement of discs in each of these zones had been estimated, it was possible to calculate that each rod produces about 80–90

discs per day. This represents a rate of about $3\frac{1}{2}$ discs per hr, or one every 16–18 min.

Pigment epithelial cells were smallest in diameter in the fovea, and were increasingly broader near the retinal periphery. Outside the fovea, the rods were disposed in circles around individual cones, with the proportion of rods to cones increasing towards the periphery. (The same relationships occur in the human retina; references 14, 20.) The outer segments of from 24 to 45 rods were inserted in the cytoplasm of each pigment epithelial cell, depending on the location within the retina.

In these young monkeys, the outer segments of the rod visual cells probably had already reached their mature dimensions.³ Assuming that growth was complete, the number of discs produced daily by each rod at the base of the outer segment (80–90) must have been balanced by the loss of a comparable number of discs at the apical end of that structure. Since the number of rods associated with each pigment epithelial cell was known (Table III), the approximate number of discs phagocytized and destroyed daily by each pigment epithelial cell could be calculated. This proved to be about 2000 discs per day in the parafovea, 3500 in the perifovea, and nearly 4000 in the periphery.

That the pigment epithelium in the rhesus monkey is indeed involved in the destruction of rod outer segment discs is apparent from the presence of groups of discs within inclusion bodies (phagosomes) in the cytoplasm of these cells. The identity of the material in the phagosomes has been demonstrated in the frog by radioautography (30).

³ Polyak (19) states that the length of rod outer segments in the central area of the rhesus monkey is $28\ \mu$, which is actually slightly less than the measured length of rod outer segments in that region in these monkeys.

FIGURE 24 Junction of the rod outer segments with the pigment epithelium. Four phagosomes, containing detached rod outer segment discs, are indicated by arrows. *n*, nucleus; *mg*, melanin granules; *er*, endoplasmic reticulum. The dense inclusion body (*x*) may contain partly digested rod discs. Electron micrograph. $\times 13,000$.

FIGURE 25 Junction of rod outer segments with the pigment epithelium. Two phagosomes containing detached fragments of rod outer segments are indicated by arrows. Note that the one on the right contains discs which have been rotated 90° from their original alignment, and now appear in cross-section. The inclusion body (*x*) appears to be a phagosome in which disc material is partially degraded. Lipofuscin granules (*lg*) may contain residues of rod outer segment digestion. *er*, endoplasmic reticulum. Electron micrograph. $\times 16,065$.

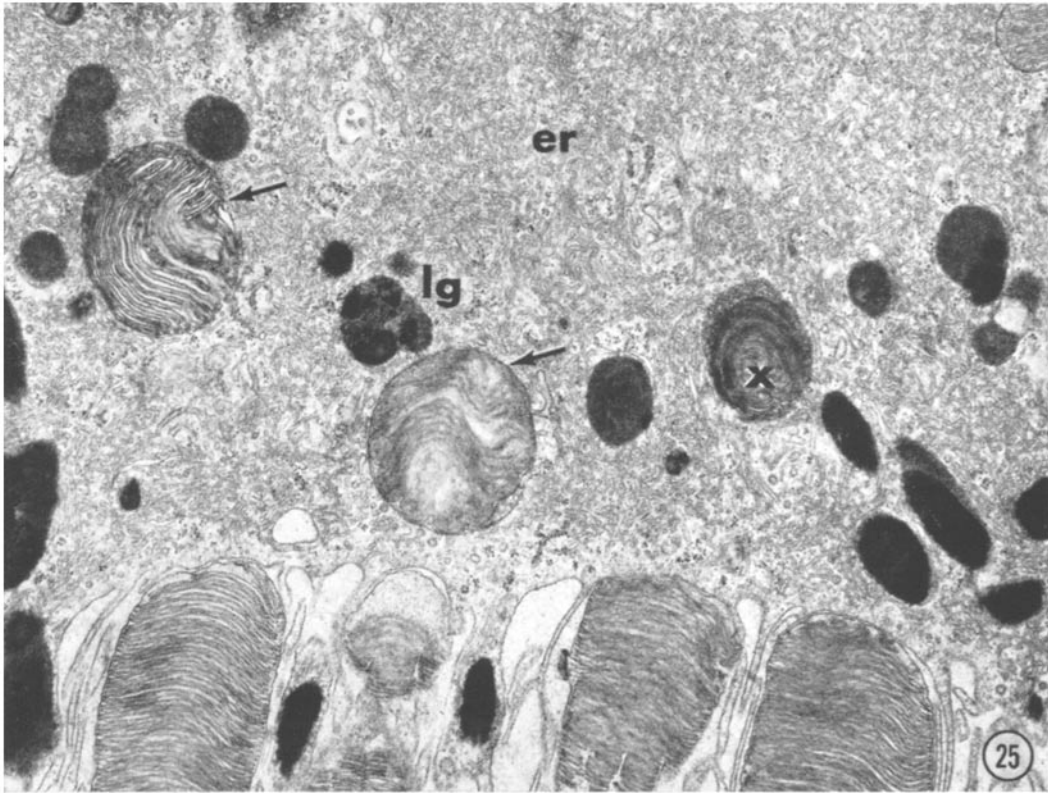
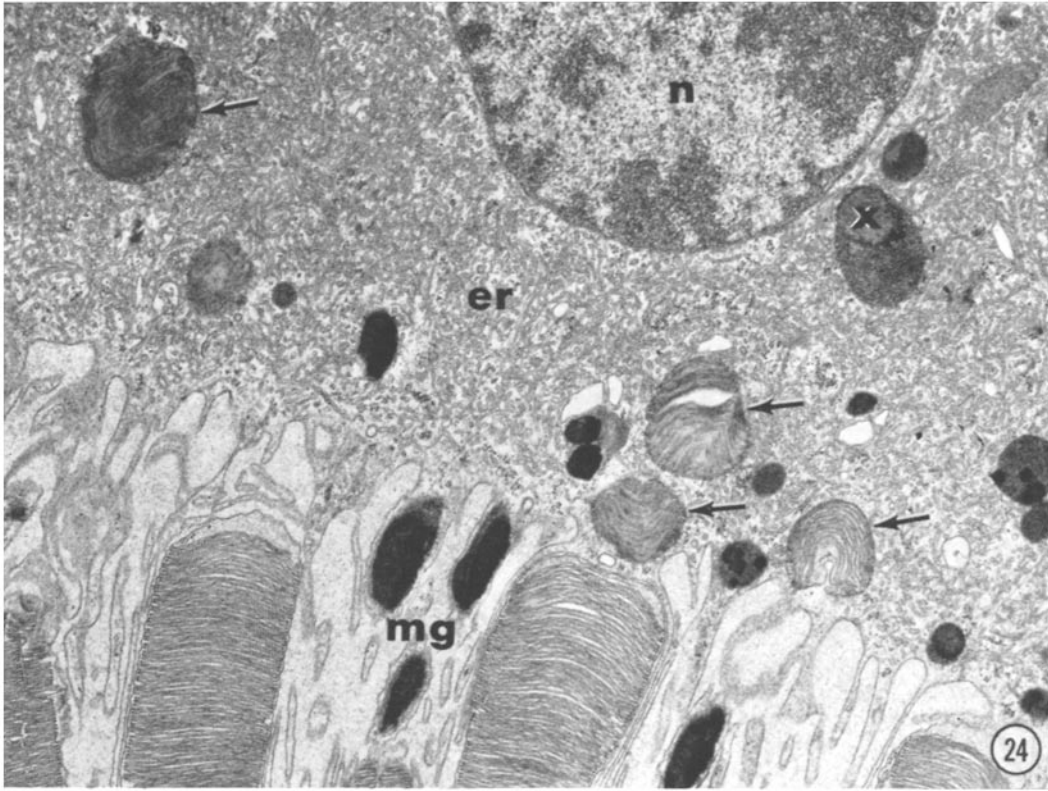


TABLE III
Distribution of Rods, Cones, and Pigment Epithelial Cells in Different Regions of the Retina

	Fovea	Parafovea	Perifovea	Periphery	Far periphery
Number of rods per 100 μ^2	None	7.3	11.0	9.9	6.8
Number of cones per 100 μ^2	19.4	1.4	0.6	0.6	0.3
Average area of pigment epithelial cell (μ^2)	186.3	336.2	404.9	425.9	567.4
Number of rods per pigment epithelial cell*	None	24.5	44.5	42.2	38.6
Number of cones per pigment epithelial cell*	36.1	4.7	2.4	2.6	1.7

* $\frac{\text{Area of pigment epithelial cell } (\mu^2)}{100 \mu^2} \times \text{number of rods or cones per } 100 \mu^2$.

Disc formation in rods is apparently a continuous process. In contrast, the exfoliation of *groups* of discs from the ends of rod outer segments indicates that the shedding process is discontinuous. A similar intermittent detachment of discs takes place in the frog (30). Morphological evidence supports the conclusion that it is the rods themselves which actively shed the discs, with the pigment epithelium serving to phagocytize the ejected membranes (29).

There was no evidence of new disc formation in cone outer segments. Had new discs been produced after the injection of the labeled amino acid, they would have contained significant quantities of radioactive protein. This would have distinguished them from discs formed before the injection (as was observed in rods). Instead, there was only a weak, diffuse distribution of new protein throughout the outer segment, comparable to that observed among the old rod discs. The presence of the diffuse reaction in cones, and the absence of any signs of new disc formation, is comparable to the situation observed previously in mature cones of the frog and salamander (26, 28).

Although a number of dissimilarities in disc ultrastructure exist between rods and cones, none of them seems to offer an explanation for the striking difference between these two classes of visual cells in the process of outer segment renewal. Discs are produced in the developing rod and cone visual cells until the mature outer segment has been constructed. Then disc formation apparently ceases in cones but not in rods. If mature cones assemble new discs in the normal retina, they must do it so rarely or so slowly that

the process has escaped detection by these radioautographic methods.

However, mature cones in the rhesus monkey retain the capacity to produce new discs if their existing disc structure is badly damaged. This is indicated by a study in which the visual cells were experimentally separated from the pigment epithelium, a condition which leads to degeneration of the rod and cone outer segments (15). When normal contact with the pigment epithelium was surgically restored, both rods and cones began to repair their outer segments by forming new discs.

Comparable structures in the human retina are strikingly similar to those of the rhesus monkey. In man, the shedding of discs from the ends of rod outer segments has also been described (1), and inclusions resembling fragments of detached rod outer segments are present in the pigment epithelial cell cytoplasm (1, 11). The dimensions of the rod outer segments are similar in both species (5, 10, 21), and in both the length decreases and the diameter increases slightly in the periphery of the retina (10, 19). In man and rhesus monkey, cone outer segments are long and thin in the fovea, and increasingly shorter and thicker towards the periphery (10, 19). Slight tapering of the outer segments of foveal cones is observed also in the human retina (10, 21).⁴ Scalloping of rod disc perimeters occurs in the monkey (4) and in man (5, 6), whereas cone discs in both are round, without scalloping (8, 17). Only the basal rod and cone discs seem to be connected to the cell membrane of the outer segment in both species (4, 5, 8, 17).

⁴ Tapering of the outer segments of the thinnest cones in the absolute center of the monkey fovea may be insignificant (8).

Swelling of the intradisc space in rods with certain fixatives, and the presence of a larger interdisc space in cones, are both known in the rhesus monkey (8, 15), and have also been documented in the human (17). The number of discs packed into a given length of outer segment is similar in the cones of monkey and man (8, 17). The disc concentration in rods also appears to be similar, although difficulties in preventing disc swelling have led to variable results (5, 8, 17). Because the lengths of monkey and human rods are similar, the number of discs per outer segment must also be very similar.

Even the visual pigment molecules in the discs of rods and cones are very much alike in man and the rhesus monkey. The single rod pigment and the three types of cone pigments which underlie the phenomenon of color vision appear to be practically identical in the two species, with regard to all characteristics which so far have been studied (23).

In the human retina, do rod outer segments undergo renewal, and does the renewal process differ in rods and cones? Present evidence suggests that the answers to these questions will prove to be affirmative. When such dissimilar species as the frog and monkey reveal comparable mechanisms of rod and cone outer segment renewal, it seems unreasonable to expect fundamental differences to exist between two species of primates, man and rhesus monkey, with such remarkably similar visual cells.

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REFERENCES

1. BAIRATI, A., JR., and N. ORZALES. 1963. The ultrastructure of the pigment epithelium and of the photoreceptor-pigment epithelium junction in the human retina. *J. Ultrastruct. Res.* 9:484.
2. BARGOOT, F. G., T. P. WILLIAMS, and L. M. BEIDLER. 1969. The localization of radioactive amino acid taken up into the outer segments of frog (*Rana pipiens*) rods. *Vision Res.* 9:385.
3. BERGERON, M., and B. DROZ. 1968. Analyse critique des conditions de fixation et de préparation des tissus pour la détection radioautographique des protéines néoformées, en microscopie électronique. *J. Microsc.* 7:51.
4. COHEN, A. I. 1961. The fine structure of the extrafoveal receptors of the Rhesus monkey. *Exp. Eye Res.* 1:128.
5. COHEN, A. I. 1965. New details of the ultrastructure of the outer segments and ciliary connectives of the rods of human and Macaque retinas. *Anat. Rec.* 152:63.
6. COHEN, A. I. 1969. Rods and cones and the problem of visual excitation. In *The Retina*. B. R. Straatsma, M. O. Hall, R. A. Allen, and F. Crescitelli, editors. University of California Press, Berkeley, Calif. 31.
7. COHEN, A. I. 1970. Rods and Cones. In *Handbook of Sensory Physiology*, Vol. 7, part IB. M. Fuortes, editor. Springer-Verlag. In press.
8. DOWLING, J. E. 1965. Foveal receptors of the monkey retina: fine structure. *Science (Washington)*. 147:57.
9. DROZ, B. 1963. Dynamic condition of proteins in the visual cells of rats and mice as shown by radioautography with labeled amino acids. *Anat. Rec.* 145:157.
10. DUKE-ELDER, S., and K. C. WYBAR. 1961. *System of Ophthalmology*. Vol. II. The Anatomy of the Visual System. The C. C. Mosby Company, St. Louis, Mo.
11. FEENEY, L., J. A. GRIESHABER, and M. J. HOGAN. 1965. Studies on human ocular pigment. In *The Structure of the Eye*. II. Symposium. J. S. Rohen, editor. Friedrich-Karl Schattauer-Verlag, Stuttgart. 535.
12. HALL, M. O., D. BOK, and A. D. E. BACHARACH. 1968. Visual pigment renewal in the mature frog retina. *Science (Washington)*. 161:787.
13. HALL, M. O., D. BOK, and A. D. E. BACHARACH. 1969. Biosynthesis and assembly of the rod outer segment membrane system. *J. Mol. Biol.* 45:397.
14. KACZUROWSKI, M. I. 1962. The pigment epithelium of the human eye. *Amer. J. Ophthalmol.* 53:79.
15. KROLL, A. J., and R. MACHEMER. 1969. Experimental retinal detachment and reattachment in the Rhesus monkey. *Amer. J. Ophthalmol.* 68:58.
16. MATSUBARA, T., M. MIYATA, and K. MIZUNO. 1968. Radioisotopic studies on renewal of opsin. *Vision Res.* 8:1139.
17. MISSOTTEN, L. 1963. L'ultrastructure des cônes de la rétine humaine. *Bull. Soc. Belge d'Ophthalmol.* 132:472.

18. PETERS, T., JR., and C. A. ASHLEY. 1967. An artefact in radioautography due to binding of free amino acids to tissues by fixatives. *J. Cell Biol.* **33**:53.
19. POLYAK, S. L. 1941. *The Retina*. University of Chicago Press, Chicago. 196.
20. SALZMANN, M. 1912. *The Anatomy and Histology of the Human Eyeball*. Trans. by E. V. L. Brown. University of Chicago Press, Chicago.
21. SCHULTZE, M. 1873. The retina. In *Manual of Human and Comparative Histology*. S. Stricker, editor. Trans. by H. Power. New Sydenham Society, London. 218.
22. SJÖSTRAND, F. S., and S. E. NILSSON. 1964. The structure of the rabbit retina as revealed by electron microscopy. In *The Rabbit in Eye Research*. J. H. Prince, editor. Charles C. Thomas Publisher, Springfield, Ill. 449.
23. WALD, G. 1969. The molecular basis of human vision. In *The Retina*. B. R. Straatsma, M. O. Hall, R. A. Allen, and F. Crescitelli, editors. University of California Press, Los Angeles. 281.
24. YOUNG, R. W. 1967. The renewal of photoreceptor cell outer segments. *J. Cell Biol.* **33**:61.
25. YOUNG, R. W. 1968. Passage of newly formed protein through the connecting cilium of retinal rods in the frog. *J. Ultrastruct. Res.* **23**:462.
26. YOUNG, R. W. 1969. A difference between rods and cones in the renewal of outer segment protein. *Invest. Ophthalmol.* **8**:222.
27. YOUNG, R. W. 1969. The organization of vertebrate photoreceptor cells. In *The Retina*. B. R. Straatsma, M. O. Hall, R. A. Allen, and F. Crescitelli, editors. University of California Press, Los Angeles. 177.
28. YOUNG, R. W. 1971. An hypothesis to account for a basic distinction between rods and cones. *Vision Res.* In press.
29. YOUNG, R. W. 1971. Shedding of discs from rod outer segments in the Rhesus monkey. *J. Ultrastruct. Res.* **34**:190.
30. YOUNG, R. W., and D. Bok. 1969. Participation of the retinal pigment epithelium in the rod outer segment renewal process. *J. Cell Biol.* **42**:392.
31. YOUNG, R. W., and D. Bok. 1970. Autoradiographic studies on the metabolism of the retinal pigment epithelium. *Invest. Ophthalmol.* **9**:524.
32. YOUNG, R. W., and B. Droz. 1968. The renewal of protein in retinal rods and cones. *J. Cell Biol.* **39**:169.