THE ROLE OF THE PIGMENT EPITHELIUM IN THE ETIOLOGY OF INHERITED RETINAL DYSTROPHY IN THE RAT

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ABSTRACT

Visual cell outer segment renewal was studied in eyes of mutant Royal College of Surgeons (RCS) and Sprague-Dawley (control) rats by a combination of microscopy and radioautography with the light and electron microscopes. RCS and control rats were injected with amino acids-^{3}H at 11 days of age. Radioactive rod outer segment discs were assembled at the outer segment base from radioactive proteins synthesized in the rod inner segments. In controls, all radioactive discs assembled at 11 days of age were displaced the length of the outer segments, removed from outer segment tips, and phagocytized by the pigment epithelium by 8 days after injection. In the RCS rats, disc assembly and displacement resembled controls for the first 3 days after injection. However, as disc assembly continued for some time thereafter, a layer of labeled, disorganized, lamellar debris accumulated between the outer segment tips and the pigment epithelium. The buildup of debris was accompanied by visual cell death. At no time during the study was there evidence for phagocytic activity by the pigment epithelium. 61 days after injection, the layer of debris was the only heavily radioactive component in the retina. In the retina of RCS rats, the outer segment renewal mechanism malfunctions because the pigment epithelium does not fulfill its normal phagocytic role. The end result is visual cell death and blindness.

Studies utilizing radioisotope techniques have recently shown that rod visual cells in the eyes of many vertebrate animals perpetually renew their light-sensitive outer segments (11, 14). This process is revealed by tracing the fate of radioactive amino acids injected into living animals. Protein, including rhodopsin (5), is continually produced in the inner segment of the cell (Fig. 1) and becomes radioactive as a result of synthesis in the presence of the labeled precursor molecules. The newly formed, radioactive protein then migrates to the base of the outer segment, where it is used in the assembly of membranous discs. An outer segment contains a stack of hundreds of such discs, and the new ones are added at the base. Due to continual disc assembly, the radioactive discs are gradually displaced toward the tip of the outer segment by newer discs containing lower levels of radioactivity. Ultimately, the intensely labeled discs are detached from the end of the cell (13). They are then phagocytized by the adjacent pigment epithelial cells (Fig. 1) within which they are degraded by intracellular digestion. Thus, in the normal rod visual cell, there is a balance between the continual assembly of new discs at the base of the outer segment and the elimination of old disc material at the tip of the cell.

Inherited retinal disorders that result in blindness occur in many animals, including man, due

to a selective degeneration of the visual cells. One such hereditary disorder, which exists in the rat, is characterized in its early stages by an abnormal accumulation of outer segment-like
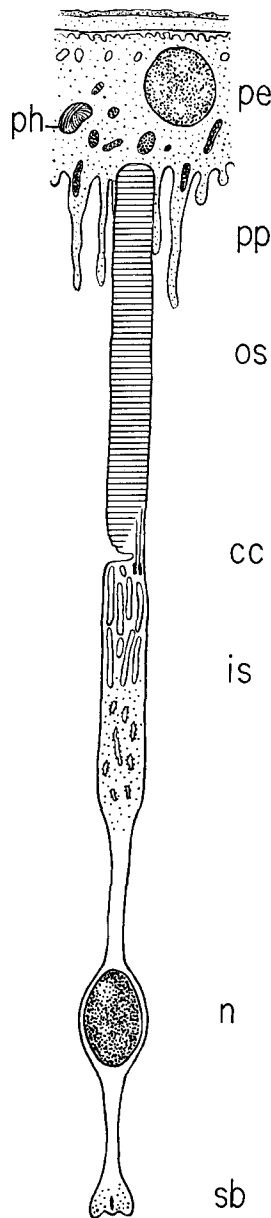

FIGURE 1 Diagram of the rat rod photoreceptor and its relationship to the pigment epithelium. *pe*, pigment epithelial cell; *ph*, phagosome within the cytoplasm of the pigment epithelium; *pp*, pigment epithelial cell processes; *os*, rod outer segment consisting of hundreds of membranous discs; *cc*, connecting structure; *is*, inner segment; *n*, nucleus located in the outer nuclear layer of the retina; *sb*, synaptic body.

membranous material at the interface between the rods and the pigment epithelium (3, 4, 9). Could this be due to an imbalance in the production and destruction of rod outer segment discs? If there is an imbalance. does it result from an over-exuberant formation of new discs by the rods, or from a failure to destroy the old ones by the pigment epithelium? Two recent reports suggest that the primary defect may reside in the failure of the scavenging function of the pigment epithelium (2, 6).

In order to explore this possibility further, rod outer segment renewal was studied in the affected strain of rats by a combination of microscopy and radioautography with the light and electron microscopes.

METHODS AND MATERIALS

10 11-day rats of the Royal College of Surgeons (RCS) strain were injected with 20 μCi/g body weight of a tritiated amino acid mixture. Half of the radioactivity in each injected dose was in L-phenylalanine (specific activity 5.1 Ci/mmole) and half in L-leucine (specific activity 58.2 Ci/mmole), both from New England Nuclear Corp., Boston, Mass. Subcutaneous injections were made into the dorsum of the neck. The animals were kept in the dark and killed at selected intervals ranging from 2 to 61 days after injection. After enucleation and removal of the cornea and lens, the eyes were immersed in a 1% formaldehyde–1% glutaraldehyde mixture in phosphate buffer, pH 7.2. Following a 2 hr fixation period, the retinas were trimmed, then postfixed in 1% osmium tetroxide for 1 hr. The retinas were dehydrated in graded ethanols and embedded in Araldite 502 (Ciba Products Co., Summit, N.J.).

0.5 μ sections were cut parallel to the long axes of visual cell outer segments and placed on glass slides. The Araldite embedment was removed from the sections with NaOH, 1/6 saturated in absolute ethanol. Some of the sections were stained with 1% toluidine blue and used for routine morphological observations. Additional preparations were dipped in Kodak NTB-2 emulsion and, following an exposure of 6.5 months, developed in Dektol (Eastman Kodak Co., Rochester, N.Y.) for 2 min at 17°C. After treatment with acid fixer, the sections were stained through the emulsion with toluidine blue.

Nine 11-day RCS rats and nine 11-day Sprague-Dawley albino rats were injected with 1 mCi/g body weight of the same amino acid mixture used above, and kept in the dark. Individuals from each group were killed at selected intervals ranging from 1.5 hr to 13 days after injection.

For animals killed at 1 day after injection or earlier, the two eyes from each animal were processed dif-

ferently than those killed at later intervals. After removal of the cornea and lens, the eyes were immersed in 4% formaldehyde in phosphate buffer, pH 7.2. Eyes from animals sacrificed at later intervals (2-13 days) were fixed in the formaldehyde-glutaraldehyde mixture described above. The 4% formaldehyde was used because it is known that labeled free amino acids, which are present in high concentrations at early intervals, are bound to protein by glutaraldehyde (1, 10). Glutaraldehyde prevented the retinal detachment which occurred with formaldehyde alone and, for this reason, was used for the later intervals. Further processing and embedding was identical to that described above. Light microscope radioautograms were prepared as described above and developed after an exposure of 4 days. Electron microscope radioautograms were prepared according to the method of Young and Droz (14), except that the preparations were developed at 18°C in Phenidone (8) (Geigy Chemical Corp., Ardsley, N.Y.) after an exposure of 3-4 months. The radioautograms were examined in a Siemens Elmiskop 1A electron microscope.

RESULTS

Development of Retinal Dystrophy by Light Microscopy

Disorganization of rod visual cell outer segment tips in RCS retinas was first perceptible by light microscopy at 13 days of age (Fig. 2). Between 13 and 16 days, the photoreceptor cells appeared normal, except for a buildup of debris at the junction between the pigment epithelium and the rod outer segment tips (Figs. 2 and 3). By 21 days, pyknotic rod nuclei were observed in the outer nuclear layer, and the outer limiting membrane appeared irregular (Fig. 4). The layer of debris between the outer segment tips and the pigment epithelium had increased in thickness. The outer segment region (which extends from the outer segment base to the pigment epithelium) was now thicker than in controls. In addition, some of the rod inner segments showed signs of swelling and degeneration. By 24 days, the number of pyknotic nuclei had increased (Fig. 5). The outer limiting membrane was less perceptible and more disorganized than at 21 days. The inner segments remained swollen and the layer of debris continued to thicken. In spite of the buildup of debris in the outer segment region, the basal portions of many outer segments remained intact. At 30 days (Fig. 6), rod nuclei began to disappear from the outer nuclear layer. Although the basal portions of many outer segments were still intact and inner segments could still be observed, the zone of intact and degenerating outer segment material,

Figures 2-10 Photomicrographs of RCS rod visual cells at intervals from 13 days to 7 months of age. 0.5 μ Araldite sections; toluidine blue. Figs. 2-9, × 800; Fig. 10, × 920.

Figure 2 13 days old. Disorganized material (bracket) between rod outer segment tips (upper arrows) and pigment epithelium (pe) can first be observed at this age in the light microscope. The outer limiting membrane (lower arrow) and outer nuclear layer (onl) can also be seen.

Figure 3 16 days old. The layer of debris (bracket) between pigment epithelium and outer segment tips (arrows) has increased in thickness.

Figure 4 21 days old. The buildup of debris continues (bracket). Visual cell inner segments (is) appear swollen, the outer limiting membrane (long arrow) is disorganized, and pyknotic photoreceptor nuclei (short arrows) are observed in the outer nuclear layer.

Figure 5 24 days old. The layer of debris (bracket) is thicker than before. Nonetheless, many outer segments (arrows) remain intact. Inner segments (is) remain swollen; the number of pyknotic nuclei in the outer nuclear layer has increased. The region between the pigment epithelium and the outer limiting membrane was thickest at this age.

Figure 6 30 days old. In spite of the buildup of debris (bracket), basal portions of intact outer segments (arrows) can still be recognized. At this age, rod nuclei began to disappear from the outer nuclear layer. The cytoplasm of the pigment epithelium appears more dense than at earlier ages.

Figure 7 44 days old. The area between the outer limiting membrane (arrow) and the pigment epithelium is completely filled with debris (bracket). The pigment epithelium is reduced in height and the outer nuclear layer (onl) is only about four nuclei in thickness. The inner nuclear layer (inl) is also visible in the field.
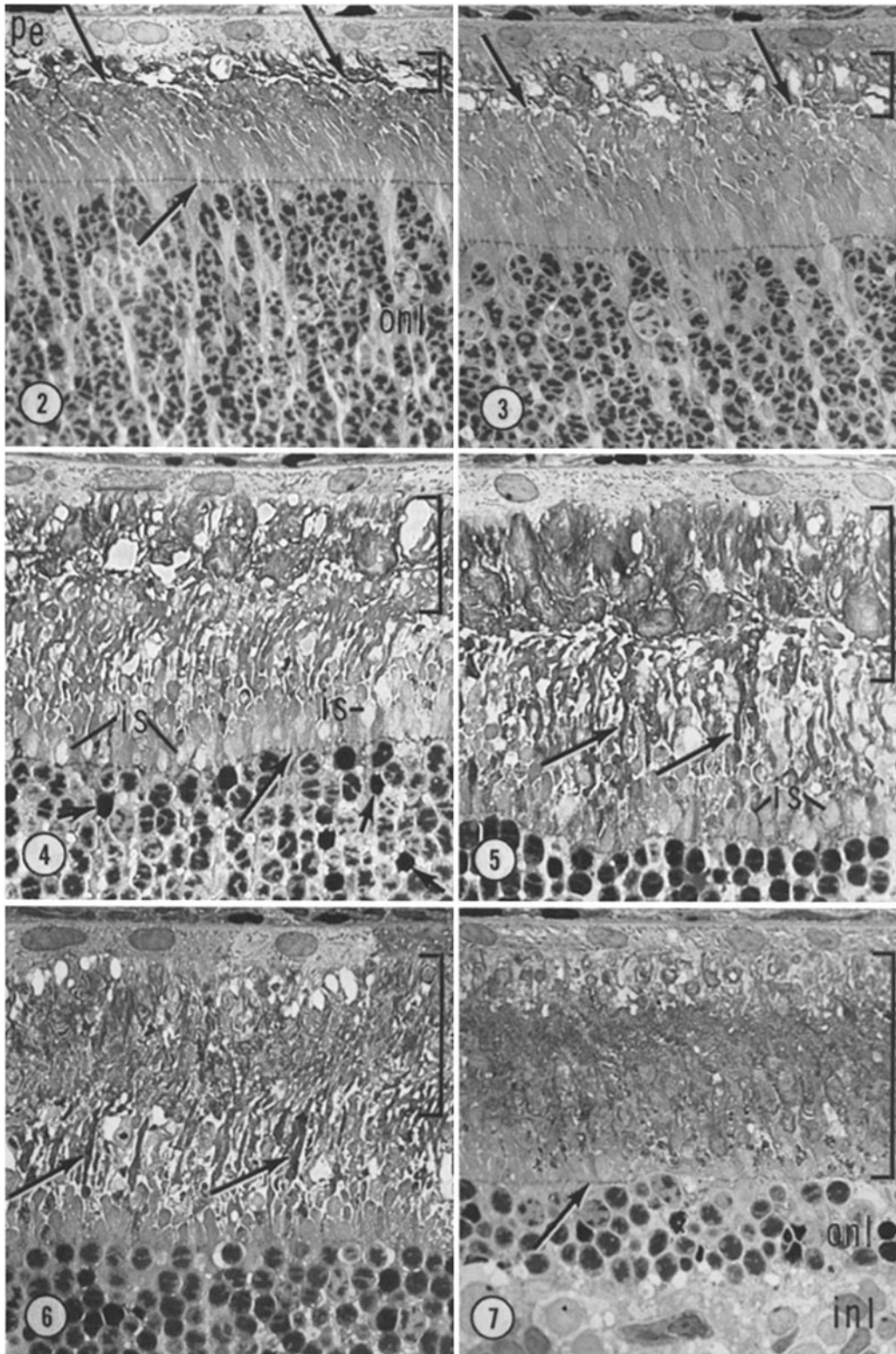
which had reached a maximum thickness at 24 days, now began to decrease in thickness. The cytoplasm of the pigment epithelium showed a greater density than at earlier intervals. By 44 days (Fig. 7), individual outer and inner segments could no longer be recognized. The entire region between pigment epithelium and outer limiting membrane was filled with disorganized material and was considerably thinner than at 30 days. The outer nuclear layer had been reduced from a layer 11–13 nuclei thick to one with a thickness of about four nuclei. The monocellular layer of pigment epithelium was also thinner than before (Fig. 7). By 58 days (Fig. 8), the outer nuclear layer was reduced to a thickness of about two nuclei. Some of the cell bodies in this layer had a larger cytoplasmic and nuclear volume than normal rod nuclei. For the first time, cells of an undetermined origin were observed in the area of compacted debris, lying near the outer limiting membrane (Fig. 8). At 72 days (Fig. 9), there was little change from 58 days. Between 44 and 72 days (Figs. 7–9), the thickness of the layer of debris between the flattened pigment epithelium and the faintly visible outer limiting membrane remained about the same. At 7 months, however, the debris had disappeared and the inner nuclear layer rested against the pigment epithelium (Fig. 10). No trace remained of the rod visual cells. The pigment epithelial layer was thicker than at any interval observed before. In addition, it

was invaded by capillaries. Evidence for gliosis could also be found (Fig. 10).

Light Microscope Radioautography

3 days after injection (earlier intervals will be described below), in both RCS and control retinas, an intense band of silver grains was oriented at right angles to the long axis of the outer segments (Figs. 11 and 12). By 5 days after injection, in control retinas (Fig. 13), the band was positioned at the tips of the rod outer segments, adjacent to the pigment epithelium. By contrast, at 5 days, in RCS retinas (Fig. 14), the band of radioactivity had shifted position in the direction of the pigment epithelium but was considerably more disorganized than at 3 days. 6 days after injection the band of silver grains began to disappear at the interface between outer segment tips and the pigment epithelium of control retinas (Fig. 15). In RCS retinas, the labeling pattern was virtually the same as at 5 days, with the exception that more of the increasingly thick layer of debris was labeled (Fig. 16). By 10 days after injection in control retinas, the intense band of silver grains was no longer visible over the outer segments. Radioactivity was evenly distributed throughout the retina (Fig. 17). From 6 days on, in RCS retinas, the band of radioactivity that had been present in the rod outer segments was gradually assimilated into the disorganized material which

FIGURE 8   58 days old. The outer nuclear layer (onl) is only two nuclei thick. Cells (short arrows) which have invaded the layer of debris can be seen on the surface of the outer limiting membrane (long arrow). The inner nuclear layer (inl) can also be observed.

FIGURE 9   72 days. The appearance of the retina is similar to that at 58 days. Cells (arrows) continue to invade the layer of debris.

FIGURE 10   7 months old. The layer of debris and outer nuclear layer observed in Figs. 2–9 have disappeared. The pigment epithelium (pe) has been invaded by capillaries (upper arrows). The inner nuclear layer (inl) is now adjacent to the pigment epithelium and is interrupted in places (lower arrows) by aggregations of Müller's cell fibers (gliosis).

FIGURES 11–18   Light microscope radioautograms of control and RCS rat retinas at various times following an injection of amino acids-³H. 0.5 μ Araldite sections, toluidine blue. × 800.

FIGURE 11   Control retina. 3 days after injection a band of silver grains (arrows) is seen over the outer segments. Radioactive protein synthesized in the inner segment has been assembled into outer segment discs. With the subsequent formation of additional discs, the most radioactive ones have been displaced for some distance along the length of the outer segments in the direction of the pigment epithelium (pe).

FIGURE 12   RCS retina. 3 days after injection, a band of silver grains similar to that in Fig. 11 is seen (arrows). A layer of debris is seen between the band and the pigment epithelium.
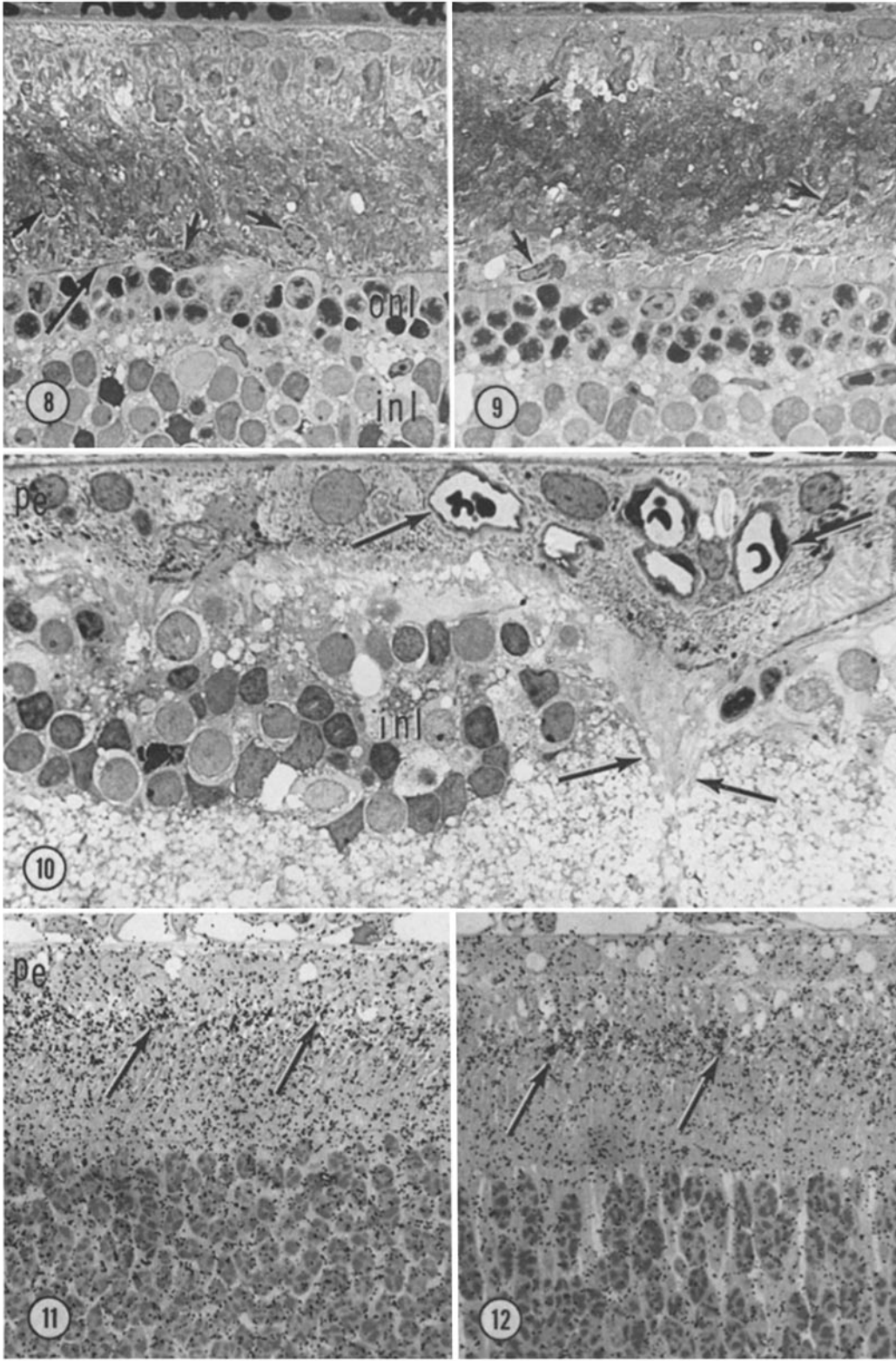
continued to fill the zone between the pigment epithelium and the outer limiting membrane. Between 6 and 22 days, in RCS retinas (Fig. 16 and Figs. 18–21), labeling patterns in the layer of debris were virtually unchanged, whereas labeling in the rest of the retina gradually declined. Between 33 and 61 days after injection (44–72 days of age), the layer of debris gradually became thinner and more compacted (Figs. 22–24), as described earlier. During this period, the most radioactive debris was located adjacent to the pigment epithelium, and the amount of radioactivity in the layer of debris gradually decreased.

Electron Microscope Radioautography

In both RCS and control retinas, protein synthesis predominated in the ribosome-rich inner segments of rod photoreceptors. This was shown in radioautograms by the localization of silver grains over that area 15 min after injection. From sites of protein synthesis in the inner segments, the labeled proteins migrated through the connecting cilium to the base of the rod outer segment where they were assembled into basal discs.

1 day after injection, in RCS and control retinas, the most heavily radioactive components of the retina were basal outer segment discs (Fig. 25). Due to technical difficulties in preparing specimens with undistorted outer segments in both groups at this age, it was impossible to make accurate comparisons between disc displacement rates in RCS and in control retinas. However, the labeling patterns were similar in both. Outer segment discs situated nearest the inner segments (basal discs) were labeled, but those near the

pigment epithelium were not. There was in the RCS retinas, however, evidence for the early accumulation of some labeled degenerating debris that was not observed in the controls.

3 days after injection, in RCS and control retinas, intensely radioactive discs were positioned two-thirds to three-quarters of the distance between the outer segment base and the tip. In control retinas, there was already some evidence of phagocytic activity by the pigment epithelium at this age (14 days). Labeled and unlabeled groups of discs were occasionally observed in the extracellular space between outer segments and pigment epithelium, or in phagosomes within the cytoplasm of the pigment epithelium itself. In RCS retinas, labeled intact outer segments were interrupted at their ends by a mass of labeled, disorganized lamellar material (Fig. 26). 5 days after injection, outer segments in control and RCS retinas had grown considerably in length. In control retinas, heavily labeled discs were positioned either a short distance from the outer segment tips or directly at the tips (Fig. 27). In addition, labeled and unlabeled groups of detached outer segment discs were frequently observed in the extracellular space or in the cytoplasm of the pigment epithelium (Figs. 28 and 29). In RCS retinas, intensely labeled discs were positioned farther from the ends of the outer segments than in controls (Fig. 30), but at an approximately equal distance from the base. The amount of labeled debris between outer segment extremities and the pigment epithelium had increased over earlier intervals.

6 days after injection, most of the outer segment tips in control retinas were heavily radio-

FIGURE 13   Control retina. 5 days after injection, the migrating band of radioactivity (arrows) has approached the interface between the rod outer segment tips and the pigment epithelium.

FIGURE 14   RCS retina. 5 days after injection, the band of silver grains has become somewhat disorganized, and has shifted slightly toward the pigment epithelium.

FIGURE 15   Control retina. 6 days after injection the transverse band of silver grains has begun to disappear at the interface between the rod outer segment tips and the pigment epithelium (arrows).

FIGURE 16   RCS retina. 6 days after injection the band of radioactivity has become further dispersed and associated with the layer of debris (bracket).

FIGURE 17   Control retina. 10 days after injection, the band of silver grains is no longer visible. The label is equally distributed throughout the retina.

FIGURE 18   RCS retina. 10 days after injection, the labeling patterns remain virtually unchanged from 6 days.
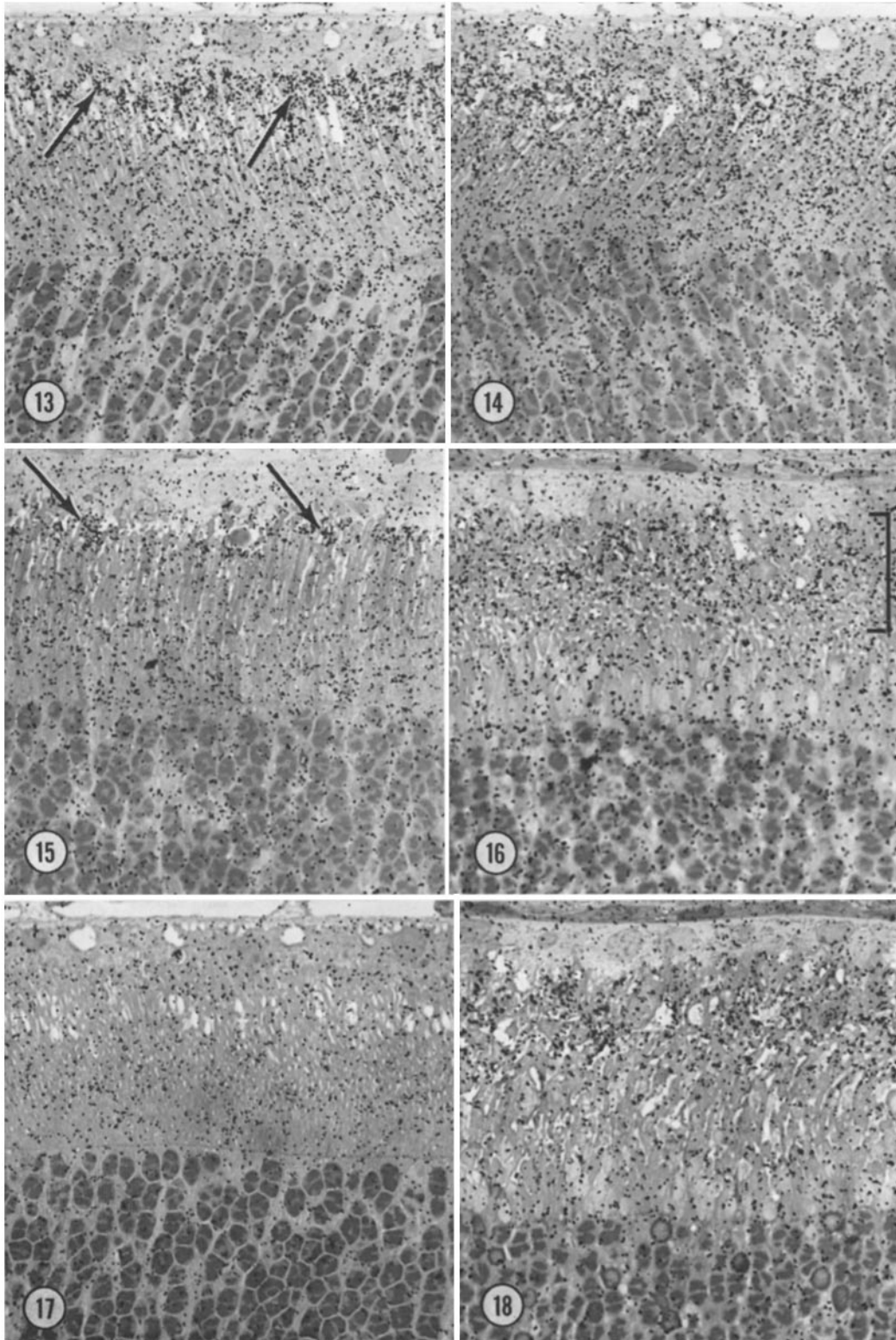
active, and numerous labeled phagosomes were observed in the pigment epithelium (Fig. 31). In RCS retinas, the most intense label was in outer segment extremities or in the debris between the extremities and the pigment epithelium (Fig. 33).

By 8 days, all of the intensely radioactive discs had been shed from the ends of the outer segments in control retinas (Fig. 32). Labeled phagosomes containing discs in various stages of digestion were observed in the pigment epithelium. From 7 to 13 days, in RCS retinas, the lamellar debris became increasingly labeled. At 13 days after injection (the longest interval studied with electron microscope radioautography), the extracellular debris was the most heavily labeled component in the retina (Fig. 34). The appearance of fragments of debris ranged from whorled lamellar patterns to dense amorphous masses. The compacted debris occupied about half the zone between the pigment epithelium and the outer limiting membrane, and in some instances was observed tightly packed between apical processes of the pigment epithelium. At no time during the intervals studied were phagosomes observed in the pigment epithelium of RCS rats. In one specimen (out of a total of 25), at 12 days after injection when the debris layer was still increasing, abnormally large, whorled clumps of lamellar debris appeared to reside within the pigment epithelium. These might have represented extracellular debris forced between adjacent pigment epithelial cells by pressure generated during the accumulation of this material.

DISCUSSION

The general pattern of protein synthesis in the inner segment from labeled amino acid precursors, and the subsequent migration of that labeled protein to the outer segment, is qualitatively the same in RCS and control rats. The results indicate that in both groups of animals new discs are assembled at the rod outer segment base and displaced toward the pigment epithelium by the constant addition of newer discs. Dowling and Sidman (4) have suggested that rhodopsin (and therefore, presumably, outer segment discs) is produced at a greater rate in RCS retinas than in controls. This can be neither substantiated nor denied on the basis of evidence presented here. However, whether or not rod outer segment disc membranes are produced more rapidly than in controls, the end result is the same because the pigment epithelium does not engulf outer segment discs.

The pigment epithelium in control animals was actively phagocytizing outer segment fragments at 14 days of age. Thus, the outer segment renewal mechanism is operative in normal animals long before the outer segment reaches its mature length. This implies that disc production exceeds disc removal for a considerable period of time (outer segments reach their mature length at 30 days of age) until a balance is finally achieved. Young (12) has recently presented evidence that outer segment tips are actually *shed* by the photoreceptor cell and not pulled off by the pigment epithelium in the rhesus monkey. Shedding of a small stack of discs is followed by

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FIGURES 19–24   Light microscope radioautograms of RCS retinas at various times following an injection of amino acids-³H. 0.5 µ Araldite sections, toluidine blue. × 800.

FIGURE 19   13 days after injection. The debris layer (bracket) remains heavily labeled.

FIGURE 20   19 days after injection. The debris layer retains its radioactivity.

FIGURE 21   22 days after injection. There has been no change in labeling of the debris layer since 6 days after injection. During this period, however, labeling throughout the rest of the retina has decreased.

FIGURE 22   33 days after injection. The debris layer has become compacted; the labeled debris has been pressed closer to the pigment epithelium.

FIGURE 23   47 days after injection, cells (arrows) have invaded the layer of labeled debris.

FIGURE 24   62 days after injection, cells (arrows) continue to invade the layer of debris. With the appearance of these cells, the radioactivity has slowly decreased.
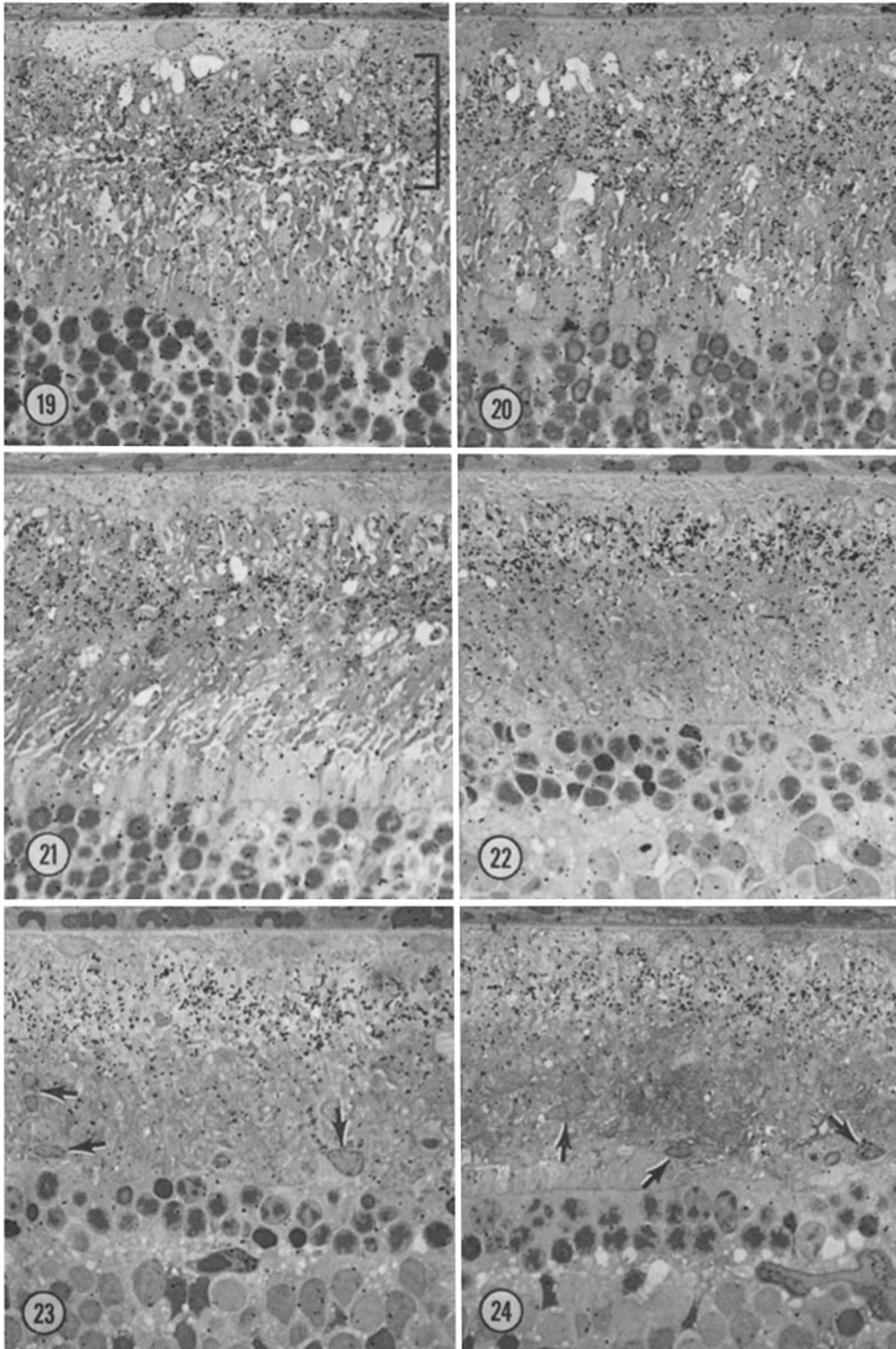
FIGURE 25–34 Electron microscope radioautograms of control and RCS retinas from rats sacrificed at intervals following an injection of amino acids-³H.

FIGURE 25 RCS retina, 1 day after injection. The most heavily labeled components were basal outer segment discs (slender arrows) which were distorted during the histological procedure. There was also some evidence for accumulation of labeled debris (heavy arrows) between the outer segments and the pigment epithelium (*pe*). Inner segments (*is*) contained moderate levels of radioactivity. × 9750.
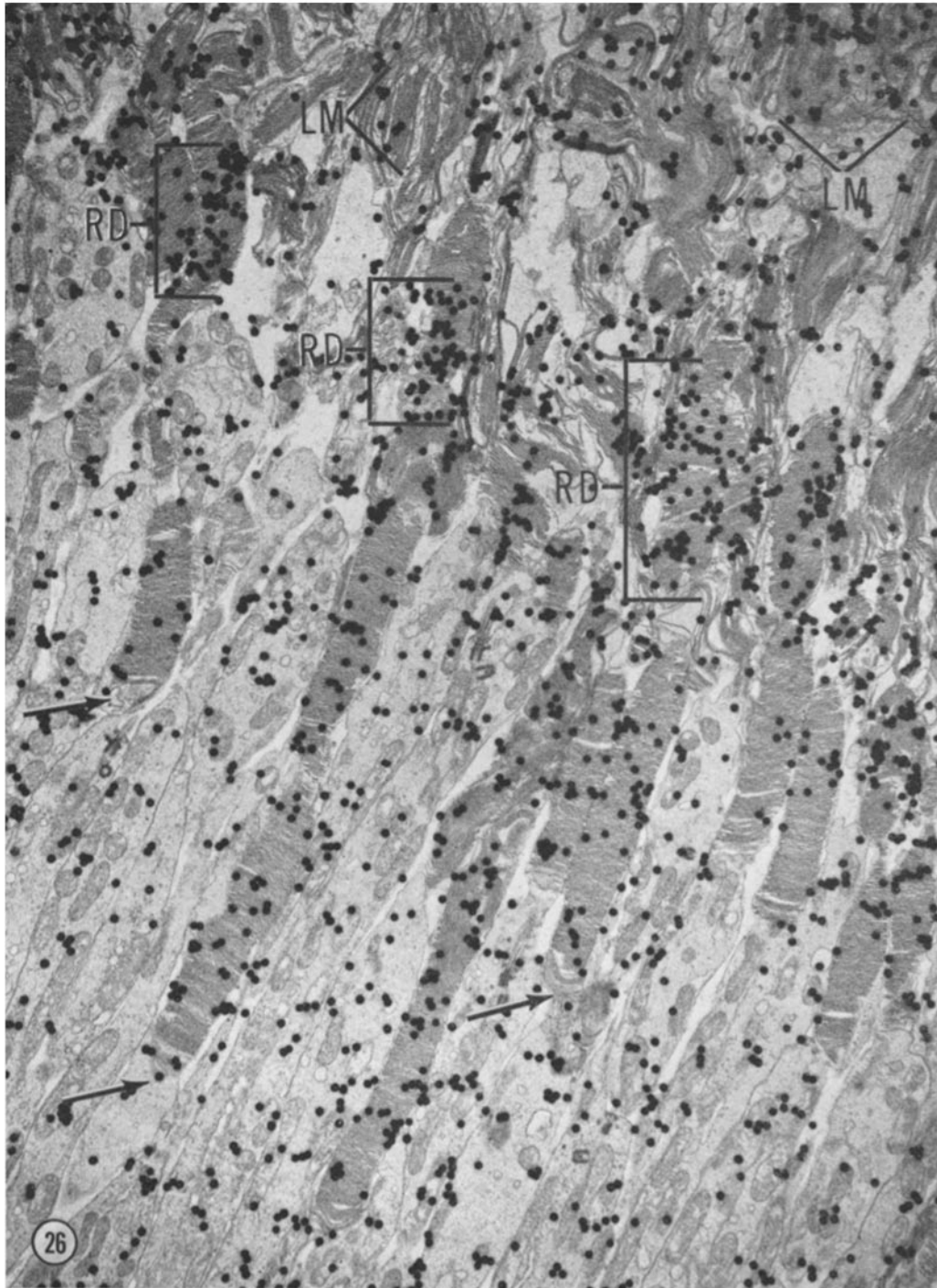
FIGURE 26 RCS retina, 3 days after injection. The most radioactive discs (*RD*) have been displaced from the outer segment base (arrows). At the top of the picture, labeled disorganized lamellar material (*LM*) is accumulating. × 6500.
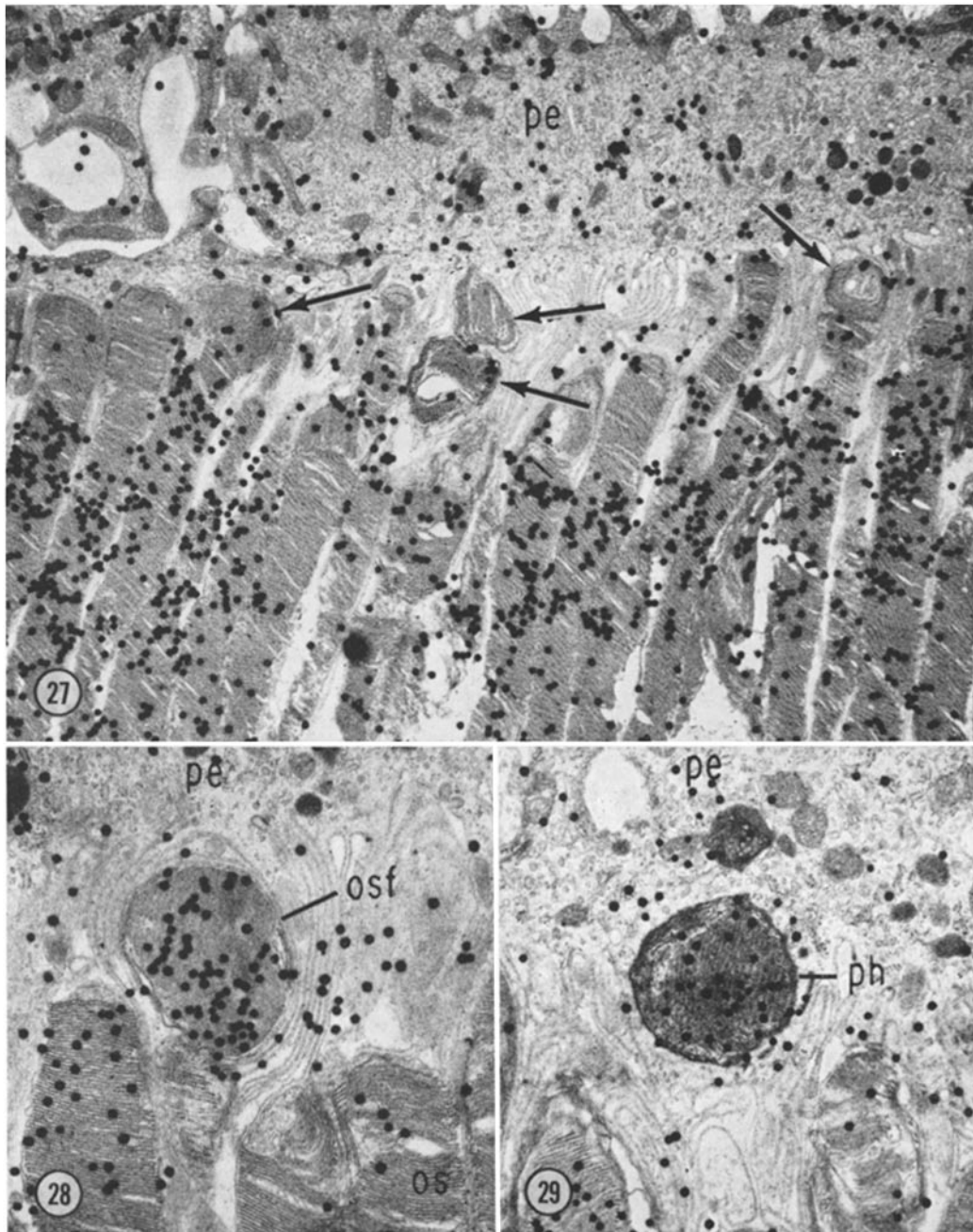
FIGURE 27 Control retina, 5 days after injection. Labeled discs are positioned at or near the outer segment tips. At this interval, and at 3 days, discs were being shed (arrows) from these extremities. *pe*, pigment epithelium. × 6500.

FIGURE 28 At 3 and 5 days after injection in control retinas, labeled outer segment fragments (*osf*) were seen in the extracellular space between outer segments (*os*) and the pigment epithelium (*pe*). × 13,000.

FIGURE 29 At 3 and 5 days after injection in control retinas, labeled phagosomes (*ph*) were also seen in the cytoplasm of the pigment epithelium (*pe*). × 13,000.
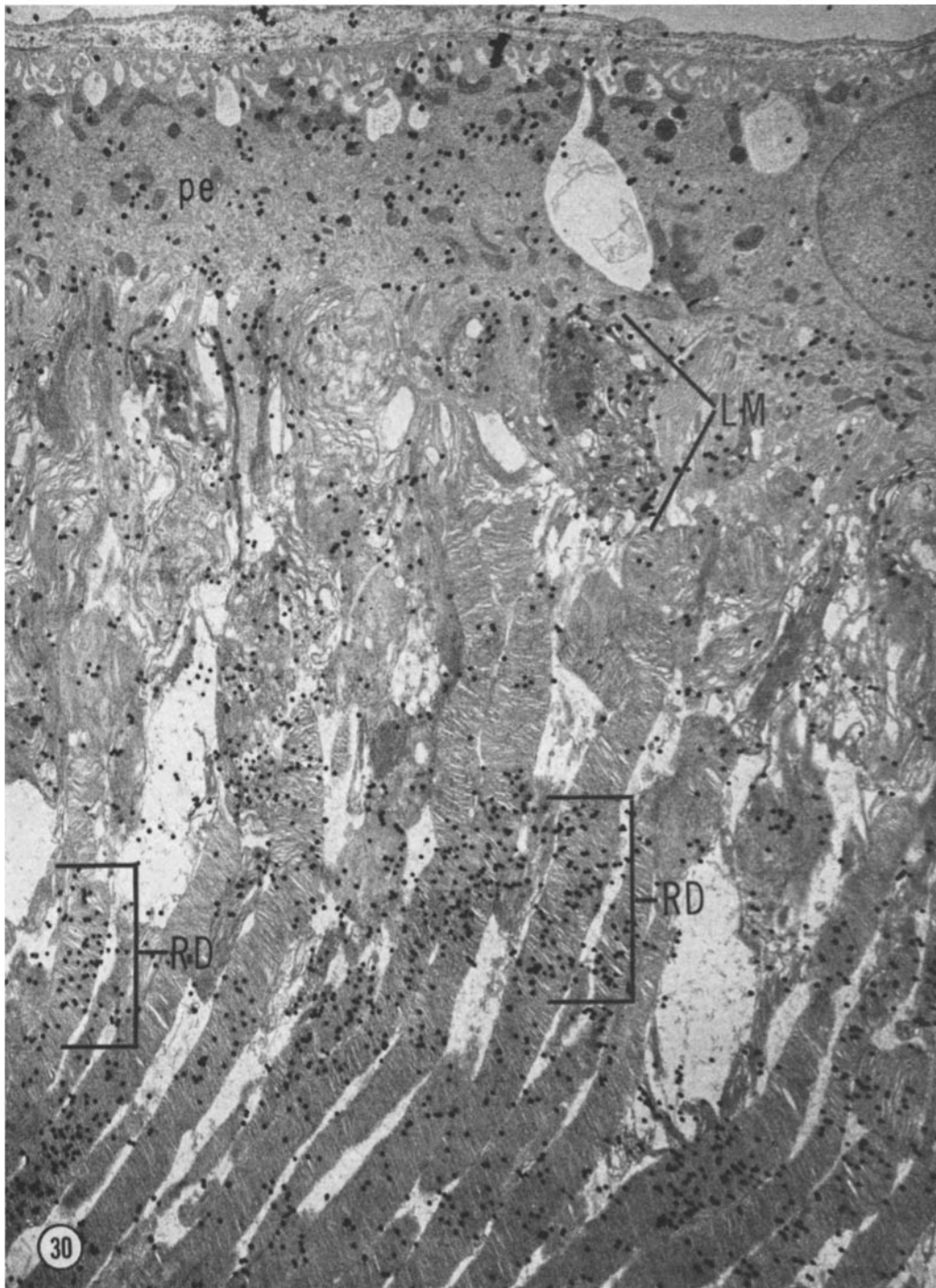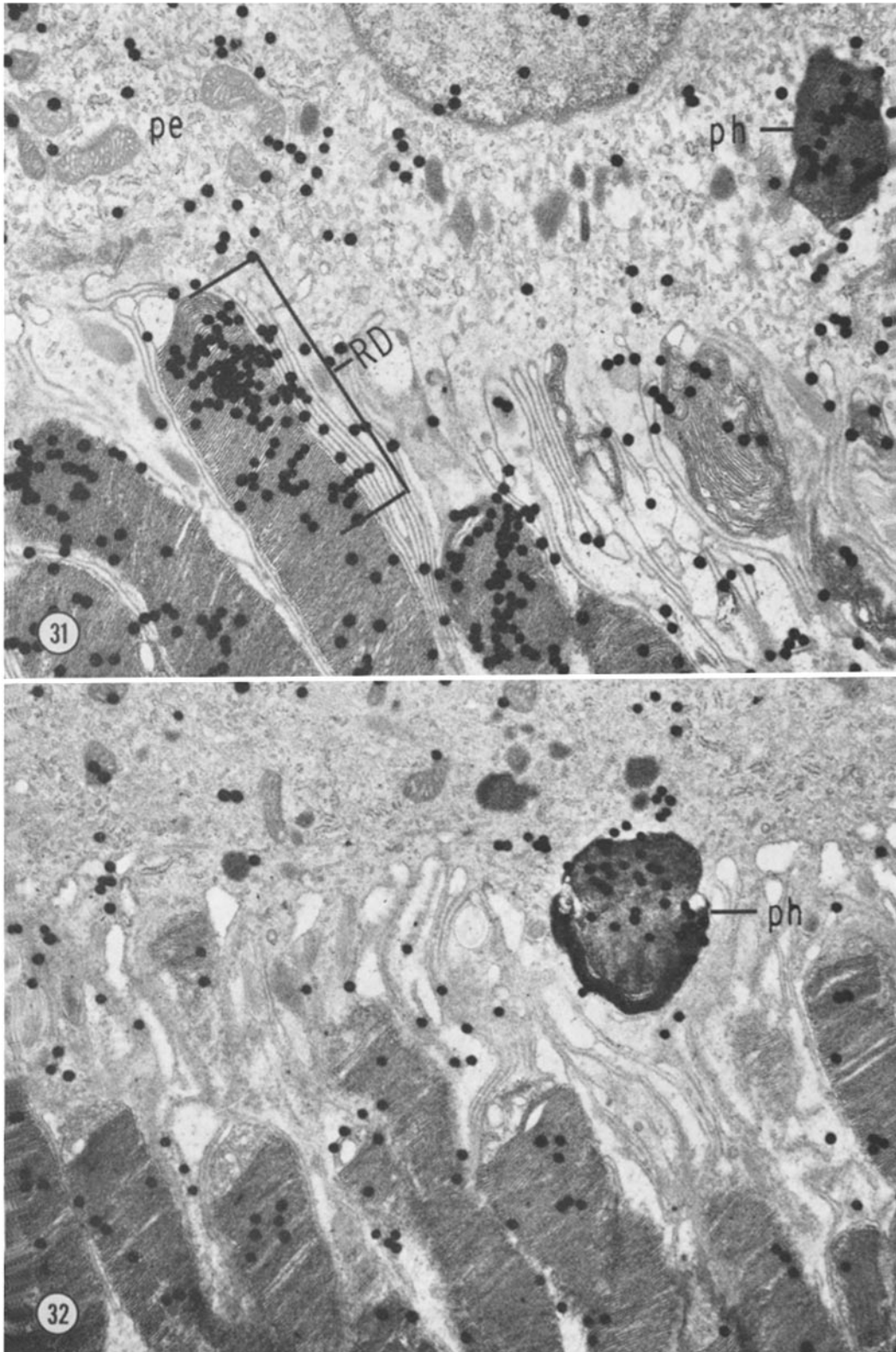
FIGURE 30 RCS retina 5 days after injection. Intensely radioactive discs (*RD*) were located at varying distances along the outer segments. Nonradioactive outer segment extremities were longer than in the controls (cf. Figs. 27 and 30). Labeled debris (*lm*) is seen between the outer segments and the pigment epithelium. No phagosomes were observed in the pigment epithelium (*pe*) at this age. × 4900.

FIGURES 31 and 32   Control retinas 6 and 8 days after injection. In Fig. 31, at 6 days, the most radioactive discs (*RD*) were seen at the outer segment ends. Labeled phagosomes (*ph*) were observed in the pigment epithelium (*pe*). × 13,000. In Fig. 32, by 8 days, all heavily radioactive discs had been removed from the outer segment extremities. Labeled phagosomes (*ph*) could still be found in the pigment epithelium. × 13,000,
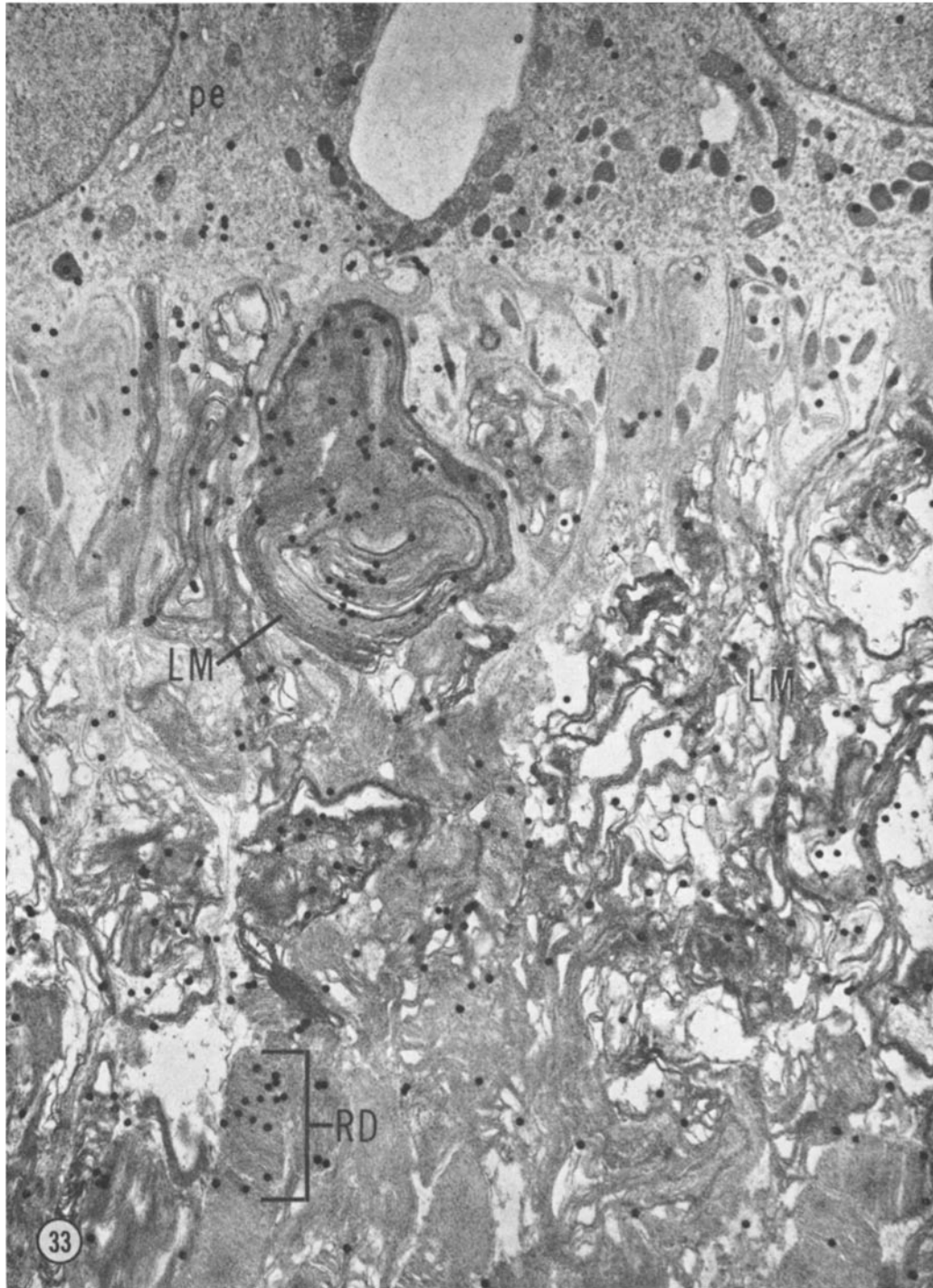
FIGURE 33 RCS retina, 7 days after injection. Radioactive discs (*RD*) are positioned at outer segment tips or have been assimilated into the layer of debris which continues to thicken. The lamellar nature of this debris (*LM*) suggests its outer segment origin. No phagosomes are seen in the pigment epithelium (*pe*). × 7650.
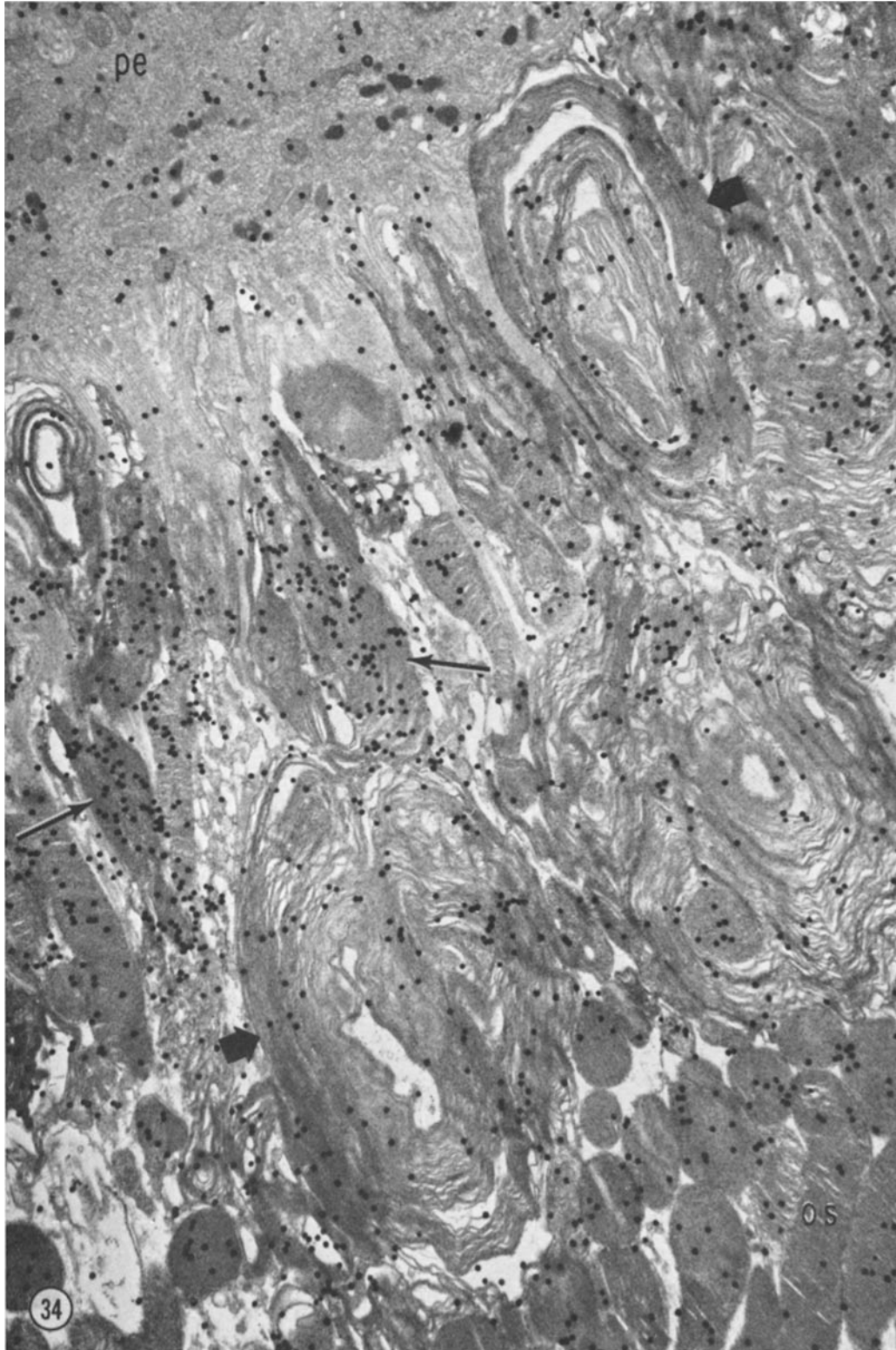
FIGURE 34 RCS retina 13 days after injection. An accumulation of debris is seen between intact outer segments (OS) and pigment epithelium (pe). Large radioactive lamellar whorls (heavy arrows) are interrupted by condensed amorphous material (slender arrows) which contain intense foci of the label. The different forms of the debris suggest a gradient in its compaction and degeneration. × 8150.

ingestion of the outer segment fragment by the pigment epithelium, with the subsequent formation of a phagosome. This implies a mechanism inherent in the outer segment extremity which controls the intermittent release of stacks of discs and the subsequent repair of the enveloping membrane of the outer segment. Secretion of substances by the pigment epithelium could also play a role in the process (12). Normal phagosomes were never observed in the pigment epithelium of RCS rats. *Thus, it would appear that the pigment epithelium in the eyes of RCS rats is not capable of phagocytizing outer segment material during the formative phases of retinal dystrophy.*

By direct electron microscope radioautographic evidence, we have shown that the pigment epithelium in RCS rats is nonphagocytic for the first 24 days of life. Herron et al. (6) arrived at the same conclusion by inference from conventional, low resolution radioautographic data. Conventional radioautographic analysis conducted over longer periods of time during this study suggests, as does the work of Herron et al., that this defect persists into adulthood.

It has previously been demonstrated that, following photoreceptor death, pigment epithelial cells are freed from their normal attachments and wander into the remaining substance of the retina (3, 4), although they are not thought to perform a phagocytic role during this time (3). During the present study, similar invading cells were observed in the layer of debris at 58 days of age. Although their appearance coincided with the gradual thinning of the debris layer, there is no indication that they were phagocytizing the radioactive debris.

Explanations for the failure of the pigment epithelium to phagocytize the degenerating outer segment disc material are speculative. It remains to be seen whether the genetic defect is expressed in the photoreceptor or in the pigment epithelial cell. The rod outer segment membranes might be abnormal in a way that prevents the pigment epithelial cells from engulfing them. There is, presently, no evidence to this effect (4). On the basis of evidence presented here, it cannot be decided for sure if the mutation results in an overproduction of rhodopsin, as suggested by Dowling and Sidman (4). It can be said with some conviction, however, that the layer of lamellar debris forms due to an *over-accumulation* of rhodopsin-rich outer segment material in dystrophic rat retinas. As a second possibility, if

the mutation is expressed in the pigment epithelium, a malfunction of the phagocytic mechanism of this cell could lead to this end result. The RCS pigments epithelium phagocytizes neither rod outer segment membranes nor india ink particles, whereas normal pigment epithelium phagocytizes both (7).

Inherited retinal dystrophy in rats and dogs and retinal aplasia in mice have been suggested as models for retinitis pigmentosa in humans. Since so little is known about the early stages of human retinitis pigmentosa and its various inherited forms, it is impossible to say whether animal models, which themselves show species differences, are well suited for this purpose. In this light, as pointed out by Dowling and Sidman (4), it is necessary to look for basic mechanisms for each phenotype before further interspecies comparisons can accurately be made. For the RCS rat, the mechanism for the basic defect at the cellular level has been demonstrated directly in the present study. The pigment epithelium in RCS rats is incapable of phagocytizing rod outer segment fragments. As the visual cells continually produce outer segment discs, the latter gradually gather in excessive amounts and an abnormal layer of debris accumulates. The question whether the mutant gene is expressed in the photoreceptor cell, in the pigment epithelial cell, or in both, remains to be answered.

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