Submicroscopic Organization of Retinal Cones of the Rabbit*

By EDUARDO DE ROBERTIS, M.D., and ARNALDO LASANSKY, M.D.

(From Instituto de Anatomía General y Embriología, Facultad de Ciencias Médicas, Buenos Aires, Argentina)

PLATES 376 TO 379

(Received for publication, May 26, 1958)

ABSTRACT

The fine structure of the cone cell of the rabbit is described and compared wtih that of the rod. The cone outer segment consists of a pile of flattened sacs with two membranes 30 A thick and a regular clear space in between of about 30 A. The membrane of the rod sacs is slightly thicker (~40 A) and the clear space is less regular and frequently absent in the deeper regions. The distance between sacs is from 85 to 95 A in the cone and from 110 to 120 A in the rod, and the total repeating period is about 190 A and 210 A, respectively. These results are discussed in relation to the concentration of solids in both photoreceptors.

A connecting cilium was observed in the cone cell and compared with that previously described in rods (4). This finding suggests that morphogenetically the cone may also result of the differentiation of a primitive cilium (5).

The inner segment of the cone shows a distal portion with large concentration of elongated mitochondria and a proximal one with a large Golgi complex in the axis surrounded by components of the endoplasmic reticulum. It is concluded that both photoreceptors have a similar general plan of submicroscopic organization, with some minor difference in fine structure probably related to their specific chemical composition and function.

Recent studies on the submicroscopic morphology of photoreceptors (2, 4–6, 8, 11–14, 19, 20) refer mainly to the rod cells, while very little has been published on the structure of the cones (12, 14). This may be due to the fact that in mammalian retinas there are fewer cones in relation to rods and the cone outer segment is extremely fragile and difficult to observe in good conditions of preservation.

Sjöstrand (12) described the outer segment of the cone in the perch as made up of many superimposed discs, which he interpreted as formed by single membranes. This was at variance with his finding of "double membrane discs" in the rod outer segment. However, in a more recent publication (14) he describes the cone outer segment of the toad as having a structure similar to that of the rod, although of different dimensions. The synaptic region of the cone with the complex relationship of the enlarged ending and the bipolar cell, has been described by one of us in the rabbit (6). In that study, physiological changes taking place in the synaptic vesicles of rod and cones were observed in animals maintained in complete darkness.

In the available literature there are no data on other regions of the cone cell or on the inner segment and its connection with the outer segment. This point is of considerable interest since it has been demonstrated in the rod cell, that this connection is of ciliary nature (4) and that the entire outer segment develops from a primitive cilium (5).

The similarities and differences in submicroscopic morphology between photopic and scotopic receptors may be of importance for a better understanding of the physiology of photoreceptors (see 16). In this paper some of the gaps in the literature are filled by analyzing the ultrastructure of the cone outer segment in the rabbit, by demonstrating the ciliary nature of the connection between the outer and inner segment, and by describing the fine structure of the cone inner segment.

* Supported by Grant B 1549 from the United States Public Health Service.

Techniques

Retinas of adult rabbits were used. Under nembutal anesthesia an incision was made beyond the sclerocorneal limbus, and after extraction of the vitreous the fixative was dropped directly into the ocular cavity. In this way most of the fixation was made *in situ* while maintaining the nervous and vascular connections. After extraction of the eye, pieces of retina were put into fresh solution of fixative at 0°C The osmium tetroxide–potassium dichromate solution of Dalton (3) and an isosmotic osmium fixative containing polyvinylpyrrolidone and balanced ions (15) were used. The pieces of tissue were properly oriented so as to obtain sections perpendicular to the surface of the retina and after embedding in butylmethacrylate were cut with a Porter-Blum microtome. The sections were observed with an RCA2E microscope with a 250 μ aperture in the condenser and a 50 μ in the objective. The residual astigmatism was compensated with a canalco stigmator.

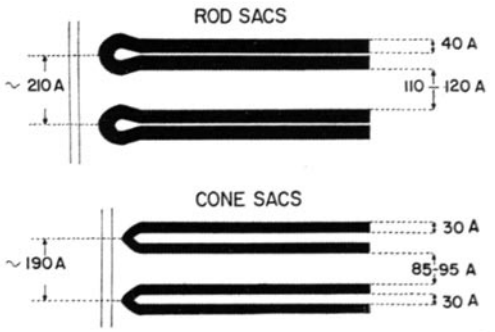
OBSERVATIONS

Present description corresponds to cones of the paracentral and equatorial zones of the retina and will encompass the following regions: the outer segment, the connecting cilium, and the inner segment.

Outer Segment.—This region of the cone cell seems to be extremely fragile and difficult to preserve even with the best fixation conditions thus far devised and in specimens in which the structure of the rods appears remarkably intact. Frequently the surface membrane is detached and fragmented and the cone sacs are swollen and dissociated. In Fig. 2, for example, most of the clear spaces may be interpreted as artifacts, while in Figs. 3 and 4 there is good preservation of the structure.

The outer segment of the cone consists of a pile of flattened sacs formed by two dense membranes of about 30 A in thickness, separated by a very regular and constant space of lesser density which is also 30 A thick (Figs. 2 to 4 and Text-fig. 1). When the sac becomes swollen, the two membranes are separated and a real cavity appears between them. At the edge of the segment the two membranes of the sac are continuous (Fig. 2 and Text-fig. 1). Each sac is separated from the neighboring ones by a clear space and no connections between them or with the surface membrane are observed.

Although in general lines the structure of cone sacs appear to be similar to that of the rod sacs, there are differences of detail that may be of importance (see Figs. 2 to 6, and Text-fig. 1). The



TEXT-FIG. 1. Diagrammatic representation of the structure of rod and cone sacs at a magnification of 1,000,000, showing the mean dimensions observed (see description in the text).

membrane of the rod sacs is slightly thicker (about 40 A). The space in between is less regular and is frequently absent in some regions of the sac. In Figs. 5 and 6 it can be observed that the clear space is evident near the surface membrane, but disappears in the inner regions. At the edge, the space between membranes becomes larger and the profiles of the rod sacs end in buttonhole-like dilatations (Figs. 5 and 6 and Text-fig. 1). This detail becomes more striking when cone and rod sacs are compared. In the latter the clear space is very regular and tapers down at the edge. The clear spaces between sacs show also quantitative differences. From several measurements, figures of 85 to 95 A for the cone and of 110 to 120 A for the rod were obtained. The net result of all the measurements gives a repeating period of about 190 A for the cone, and of about 210 A for the rod outer segment (Text-fig. 1). Near the proximal end, the regular membranous structure of the cone outer segment is replaced by a region in which the periodicity is lost and the flattened sacs may be replaced by clear vesicles of varying dimensions (Figs. 1 and 7).

In this study a large number of particles of reduced osmium were found throughout the entire outer segment of rods and cones and in the mitochondria of the inner segment. The significance of these precipitates will be discussed later.

Connecting Cilium.—Between the outer and the inner segment of the cone there is a connecting cilium (*CC*) whose structure is very similar to that previously described for rod cells (4). However this connection is more difficult to find in cone sections because the mass of the inner segment is much larger than in the rod, and the cilium is shorter (Figs. 7 and 8). In favorable longitudinal

sections (Fig. 7) the *CC* appears as a truncated cone with the base pointing toward the outer segment. The short stalk is surrounded by a surface membrane in continuity with that of the inner and outer segments and is formed by a bundle of longitudinal filaments embedded within the matrix. The proximal portion of the cilium is continuous with the inner segment, and the filaments end in a region of higher electron density, probably corresponding to a basal body. It has not been possible to observe two distinct basal bodies as in the *CC* of the rod (Fig. 8). The distal end of the *CC* penetrates deeply into the outer segment (Fig. 7). In this region the filaments are difficult to follow and only the matrix with an amorphous or granulous material can be observed.

Inner Segment.—The inner segment of the cone is very large and exceeds in length the corresponding segments of the neighboring rods (Fig. 1). As in the rod (4), a distal and proximal region may be described. In the distal portion there is a large concentration of elongated mitochondria forming the so called ellipsoid. The orientation of mitochondria is not regularly longitudinal as in the rod (4). The surrounding cytoplasmic matrix is very abundant in the cone and contains numerous clusters of dense particles, but few membranous elements of the endoplasmic reticulum (Fig. 1). The proximal region of the inner segment shows few mitochondria. Along the axial region of the segment there is a voluminous Golgi complex (Fig. 9) formed by an accumulation of large vacuoles, smaller vesicles of 300 to 600 A, and few membranes. At the sides of the Golgi complex some canalicular structures of the endoplasmic reticulum surrounded by numerous dense particles can be observed. A few fine fibrils are also found within the matrix. Along the surface membrane fine glial processes arising at the level of the outer limiting membrane may be observed (Fig. 9).

DISCUSSION

The most general conclusion to be drawn from this study is that there is a single plan of submicroscopic organization for both types of photoreceptors. This similarity is manifested in every different region as between the most distal end and the synaptic junction. The outer segment of both rods and cones are built of a pile of numerous flattened sacs. This identity in the general pattern of organization agrees with the view that the basic chemical mechanisms of cone and rod vision are essentially similar (17) and confirms the close relationship existing between structure and function in photoreceptors. Furthermore the fact that in both photoreceptors there is a connecting cilium may indicate that the outer segment of the cone has a morphogenetic origin similar to that observed in the rod (5). This similarity is not surprising if one follows the classical literature on this subject (see 5).

Beyond this structural identity of organization and morphogenesis between rods and cones, there are notable differences in fine structure, which may be related to specific functional and chemical composition. The net difference in thickness of the membranes of the rod and cone sacs may explain the different concentration of solid material found by refractometry and interference microscopy. Sidman (9) calculated the concentration of solids of the rod as 40 to 43 per cent and that of the cone outer segment as about 30 per cent. This problem and its relationship with the chemical organization of the membranes is being studied.

The observation of a zone in which the cone sacs are not well oriented and appear vesicular, at the proximal region of the outer segment, is interesting in view of similar findings in growing rods which were interpreted as representing a process of active formation of new rod sacs (5).

The abundant osmium precipitate found throughout the outer segment and the mitochondria of the ellipsoid in both visual cells, is a matter of special concern, particularly because of its regular appearance in all conditions of fixation. The possibility that it is due to the strong reducing properties of SH groups (1), that are abundant in the outer segment and ellipsoid (10, 18), was ruled out by treating the tissue with *p*-chloromercuribenzoate prior to fixation. Since it is known that ethylenic linkages are abundant in the outer segment, as can be demonstrated with the performic acid-Schiff reaction (7), it remains to be proven if the reducing substances are of lipide nature. However, in view of the fact that these linkages are not found in the ellipsoid (7), this explanation seems dubious.

BIBLIOGRAPHY

1. Bahr, G. F., *Exp. Cell Research*, 1954, **7**, 457.
2. Carasso, N., *Proc. Stockholm Conference Electron Micr.*, New York, Academic Press, Inc., 1956, 192.
3. Dalton, A. J., *Anat. Rec.*, 1955, **121**, 281.
4. De Robertis, E., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 319.

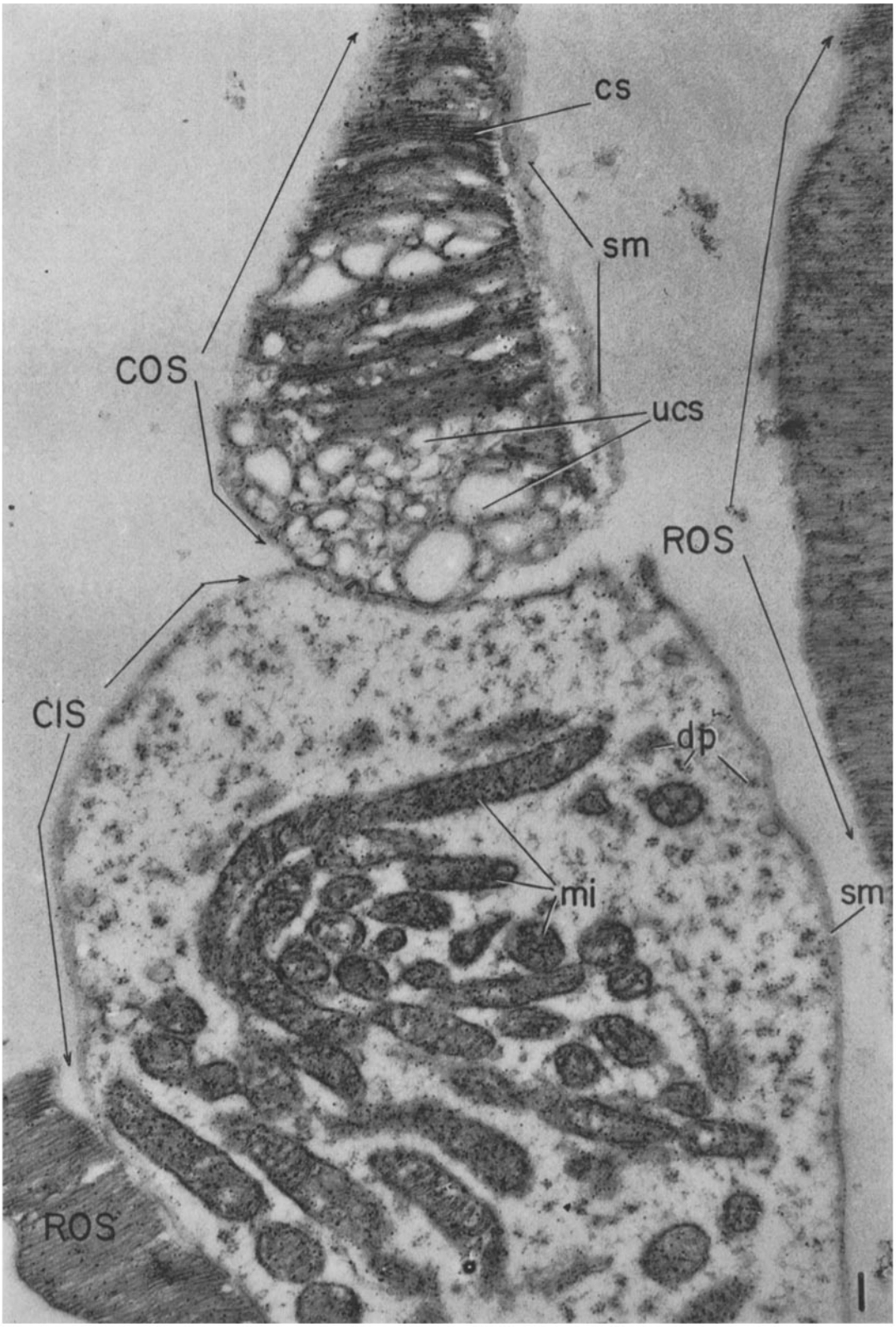
5. De Robertis, E., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** No. 4, suppl., 209.
6. De Robertis, E., and Franchi, C. M., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 307.
7. Lillie, R. D., *Anat. Rec.*, 1952, **112,** 477.
8. Porter, K. R., The submicroscopic morphology of protoplasm, *Harvey Lectures*, 1957, **51,** 175.
9. Sidman, R. L., *J. Biophysic. and Biochem. Cytol.*, 1957, **3,** 15.
10. Sidman, R. L., and Wislocki, G. B., *J. Histochem. and Cytochem.*, 1954, **2,** 413.
11. Sjöstrand, F. S., *J. Cell. and Comp. Physiol.*, 1949, **33,** 383.
12. Sjöstrand, F. S., *J. Cell. and Comp. Physiol.*, 1953, **42,** 15.
13. Sjöstrand, F. S., *J. Cell. and Comp. Physiol.*, 1953, **42,** 45.
14. Sjöstrand, F. S., *Proc. Stockholm Conference Electron Micr.*, Inc., New York, Academic Press, 1956, 194.
15. Trujillo-Cenóz, O., *Z. Zellforsch.*, 1957, **46,** 272.
16. Wald, G., Mechanism of vision. Nerve impulse, Transactions 4th Conference Josiah Macy, Jr. Foundation, New York, 1954.
17. Wald, G., *Am. J. Ophth.*, 1955, **40,** 18.
18. Wislocki, G. B., and Sidman, R. L., *J. Comp. Neurol.*, 1954, **101,** 53.
19. Wolken, J., *J. Cell. and Comp. Physiol.*, 1956, **48,** 349.
20. Wolken, J., *Tr. New York Acad. Sc.*, 1957, **19,** 315.

Explanation of Plates

bb, basal body.
ccc, cone connecting cilium.
cf, cilium filament.
CIS, cone inner segment.
COS, cone outer segment.
cs, cone sacs.
dp, dense particles.
er, endoplasmic reticulum.
G, Golgi complex.
gp, glial process.
i, interspace between sacs.
mi, mitochondria.
rcc, rod connecting cilium.
RIS, rod inner segment.
ROS, rod outer segment.
rs, rod sacs.
sm, surface membrane.
ucs, unoriented cone sacs.

Plate 376

FIG. 1. Electron micrograph of a cone cell of the rabbit's retina. Part of the outer segment and the distal region of the inner segment with the ellipsoid are shown. Notice the two neighboring rod outer segments (see further description in the text). × 50,000.



(De Robertis and Lasansky: Retinal cones of rabbit)

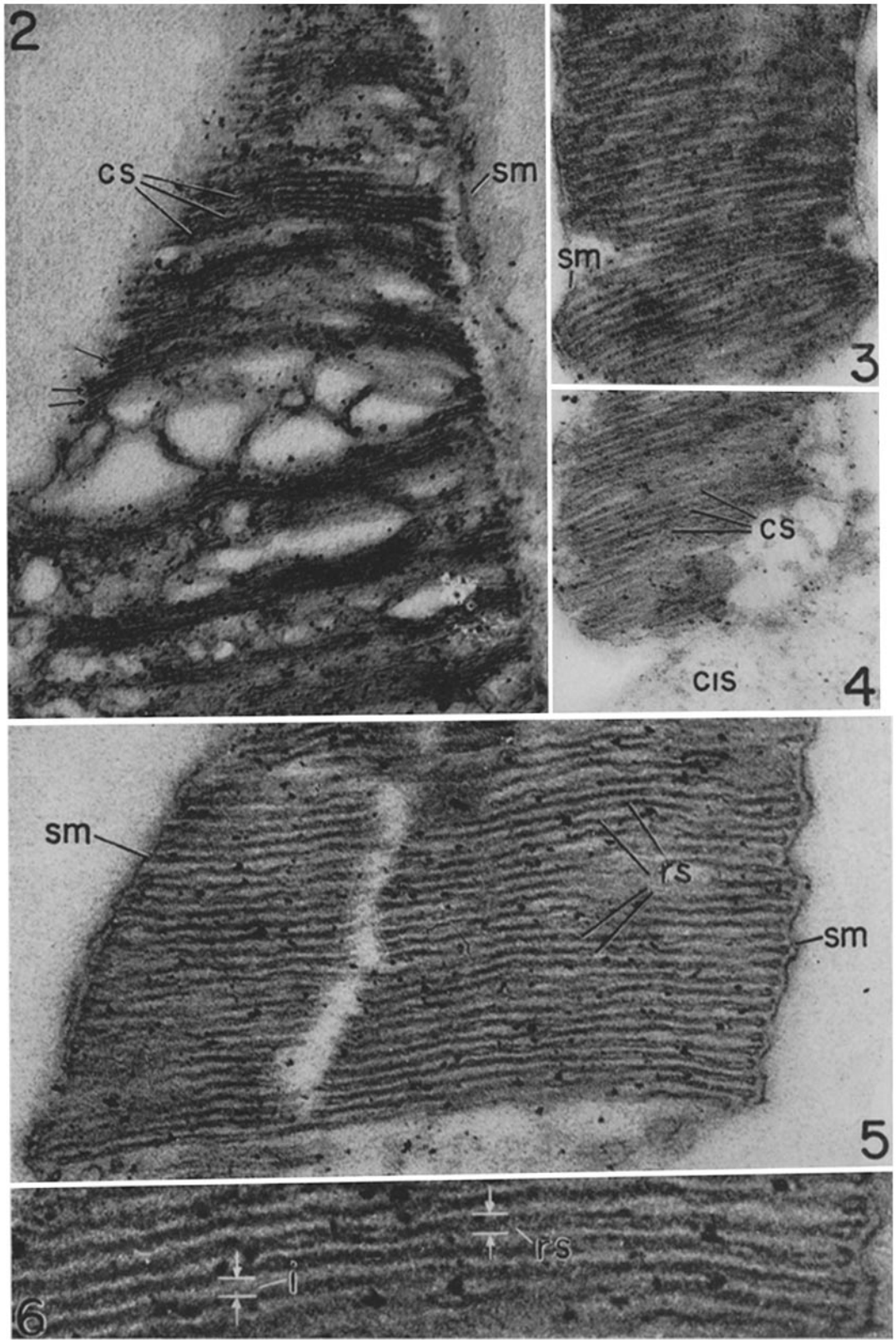
PLATE 377

FIG. 2. Enlarged portion of the outer segment of the cone shown in Fig. 1. The regular stacking of the cone sacs (*cs*) is distorted near the middle of the figure, probably by artifact. Notice the structure of the cone sacs with the clear space in between the membranes and the tapering ends (marked with arrows). × 95,000.

FIGS. 3 and 4. Portions of the outer segment of a cone, showing a better preservation of the regular piling of cone sacs (*cs*) and the surface membrane (*sm*). × 70,000.

FIG. 5. Portion of the outer segment of the rod, showing the regular stacking of rod sacs (*rs*) and the surface membrane (*sm*). × 95,000.

FIG. 6. Part of Fig. 5 at higher magnification. Notice the enlarged buttonhole-like spaces of the rod sacs at the right side and the disappearance of this space in between the membranes in the deeper region. The interspace between rod sacs is indicated at *i*. × 185,000.

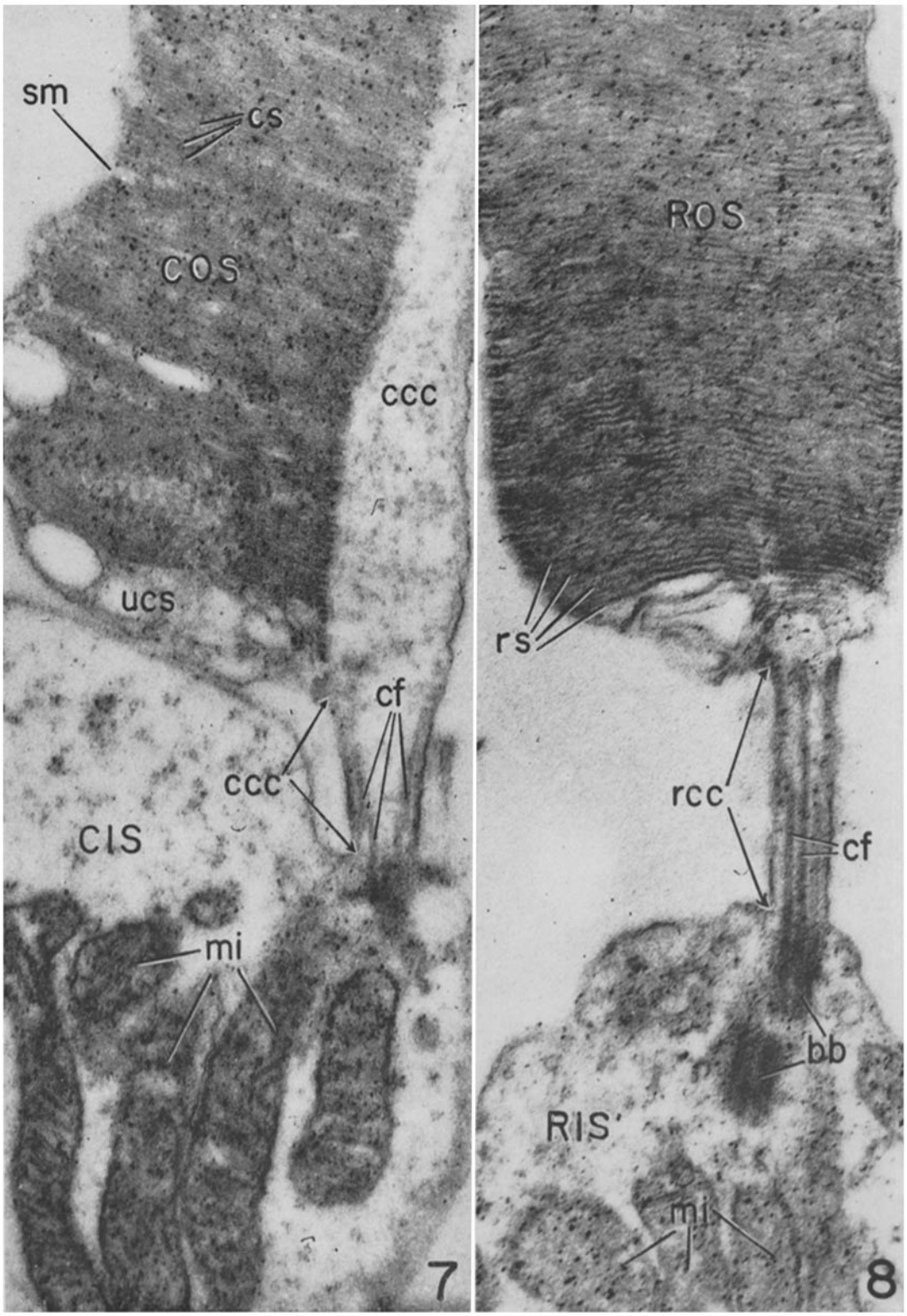


(De Robertis and Lasansky: Retinal cones of rabbit)

PLATE 378

FIG. 7. Electron micrograph of a cone cell, showing part of the outer and inner segments and the connecting cilium (*ccc*). Compare the difference in size and shape of this *CC* with that of the rod cell (Fig. 8). Some filaments of the cilium (*cf*) are indicated, and the continuation of the cilium into the outer segment is shown. The outer segment shows a proximal portion in which the structure is less oriented (*ucs*). × 40,000.

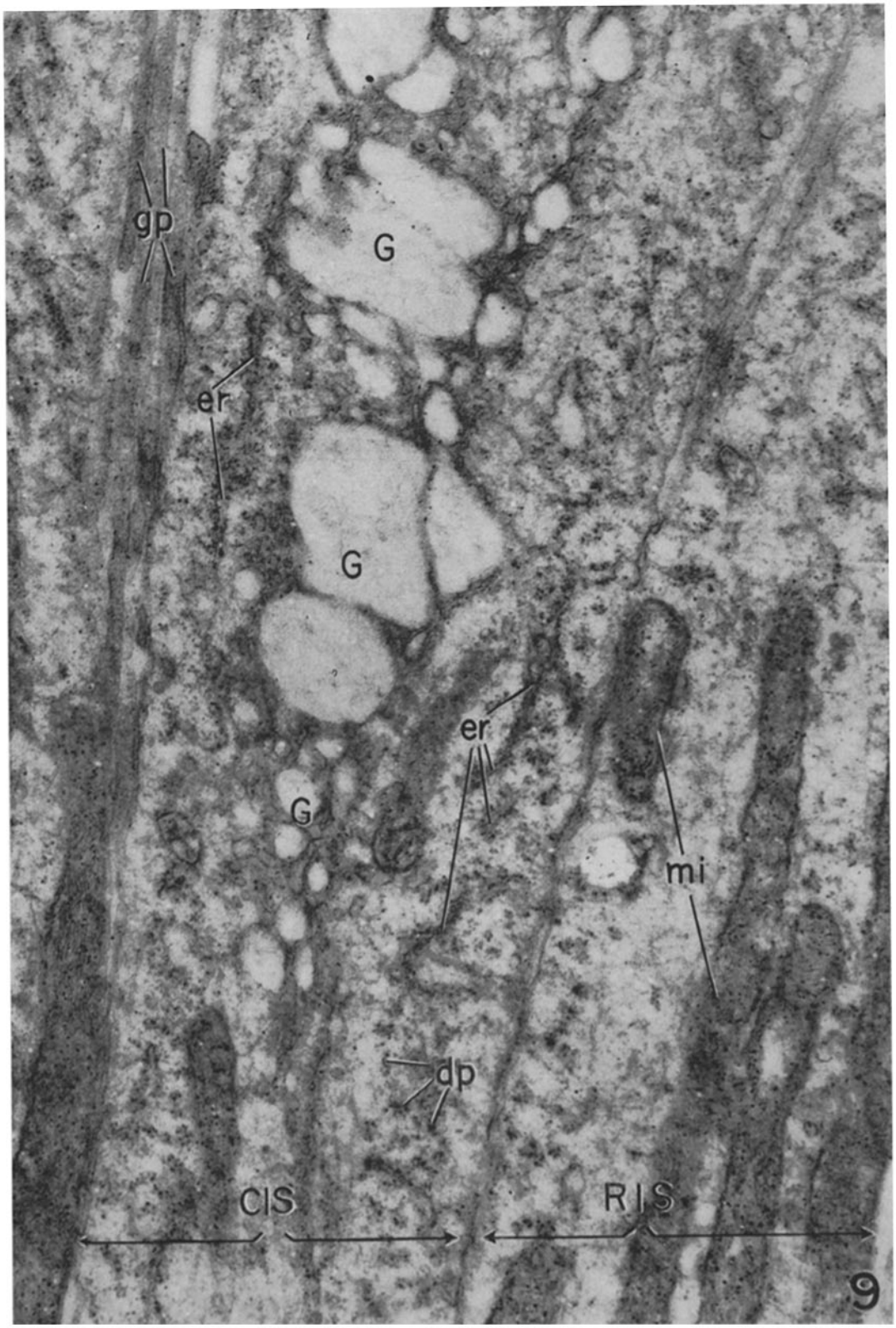
FIG. 8. Electron micrograph of a rod cell, showing part of the outer (*ROS*) and the inner segment (*RIS*), and the connecting cilium (*rcc*) with two basal bodies. × 50,000.



(De Robertis and Lasansky: Retinal cones of rabbit)

PLATE 379

FIG. 9. Electron micrograph of the proximal region of the cone inner segment, showing a large axial Golgi complex, made of large vacuoles (*G*) and smaller vesicles. On the sides are membranous components of the endoplasmic reticulum with numerous attached dense particles. See the glial processes (*gp*) in between the cone and the rod inner segment of the left. × 40,000.



(De Robertis and Lasansky: Retinal cones of rabbit)