MORPHOGENESIS OF THE RETINAL RODS

An Electron Microscope Study

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Plates 70 to 73

In a previous paper the submicroscopic morphology of the outer and inner segments of the rod cell of the adult retina has been described (1). The conclusion was drawn, that the fibrous connection between both segments is constituted by a short cilium, the so called "connecting cilium," which is composed of nine pairs of filaments, a basal body, matrix material, and a surface membrane. The outer or receptor segment of the rod, confirming the previous work of Sjöstrand (2), is composed of a pile of numerous flattened sacs or cisternae bound by a thin membrane and constituting close compartments which are probably connected by short tubular stalks to the cilium. The inner segment contains the mitochondria in the distal part (ellipsoid) and, in the proximal part, leading to the rod fiber, the Golgi substance, endoplasmic reticulum, dense particles, and neuroprotofibrils. Both segments of the rod and the connecting cilium are limited by a continuous surface membrane. It was suggested that this membrane may be the site for the propagation of the nerve impulse originated at the level of the rod sacs by the photochemical reaction. It was also postulated that the entire outer segment of the rod may be organized as a differentiation of a primitive cilium (1).

These observations and interpretations of the submicroscopic morphology of the adult rod cell made it highly interesting to study, with the electron microscope, the morphogenesis and differentiation of the inner and outer segments. For this purpose the retina of young mice was selected since at birth it is completely undifferentiated and the differentiation of the visual cells starts only after the 3rd or 4th day proceeding to completion between the 12th to the 16th day (3, 4).

In this material the development of a typical cilium and the differentiation of its apical portion to constitute the outer segment have been demonstrated. The basal portion of the primitive cilium with its basal body remains undifferentiated and constitutes the connecting cilium of the adult rod.

This study throws also some light on the macromolecular mechanisms leading to the formation of the complex lamellar structure which constitutes the receptor portion of the rod cell.

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TECHNIQUES AND OBSERVATIONS

This study was carried out on the retina of white mice from birth to the 16th day of age. The eyelids opened between the 12th and 13th days. Fixation and other preparative techniques were essentially the same as described in a previous paper (1).

The material that gave the best results, both from the point of view of fixation and of stages in the morphogenesis of the rods, belonged to mice of the 8th and 12th days after birth; for this reason our description will concentrate mainly on this material.

Until the 4th day no special signs of differentiation of the rod segments could be observed but at the 8th day this seems to proceed at a fast rate. Examination of the outer edge of the granulous layer reveals several stages in the primordium of the internal and external segments. The first change to be observed is the bulging of protoplasm beyond the external limiting membrane. This bulge is filled with numerous mitochondria and contains also a large number of dense particles, a circumscribed region of Golgi material, and a basal body. Neither the typical vacuolar elements of the endoplasmic reticulum nor the neuroprotofibrils, as observed in the adult retina, could be found. At this stage the differentiation of the internal segment into a distal part, with parallel arranged mitochondria (ellipsoid), and a proximal part is not observed. The mitochondria with their characteristic internal structure are randomly oriented and fill the entire bulge of protoplasm (Fig. 4).

Observations on the Morphogenesis of the Outer Segment

In the description of the morphogenetic changes of the primitive rod cell, leading to the formation of the outer segment, three main stages will be considered. The first corresponds to the development of the primitive cilium; the second to the rapid enlargement of the apical part of the cilium due to the formation of the rod sacs; and the third involves the rearrangements occurring in the rod sacs to constitute the typical adult outer segment.

I. The Primitive Cilium and the Morphogenetic Material

Figs. 1 and 3 illustrate two extreme stages in the development of the outer segment. The first (Fig. 1) is an early stage at 8 days in which this segment is constituted essentially by a cilium projecting from the bulge of protoplasm of the inner segment and the second (Fig. 3) is an outer segment, almost entirely differentiated at 12 days. The comparison of both figures clearly shows that the upper portion of the cilium has differentiated by the formation of the numerous lamellar sacs of the external segment. The lower portion remains undifferentiated as the "connecting cilium" of the adult rod (1). This comparison also suggests that some parts of the apical portion of the cilium persist in the differentiated segment along the right edge of it. As a matter of fact an area of about the same size as the original cilium is found which contains parts of the filaments of the cilium and a granular material. This area is bounded on one side by the surface membrane and on the other by the edges of the rod sacs

(Fig. 3, marked with arrows). This region corresponds in the adult to the series of incisures of the rod sacs, to the insertion of the filaments of the connecting cilium, and to the small tubular projections of the rod sacs (see reference 1).

Observation of the primitive cilium both in longitudinal (Fig. 1) and in cross-sections (Fig. 2) shows a characteristic difference from the structure of the connecting cilium of the adult (compare Fig. 1 of this paper with Fig. 7 of reference 1). This is indicated by the presence, within the matrix of the cilium and filling specially the enlarged apical end, of an ill-defined dense granular material. This component will be called "the morphogenetic material" of the rod because we believe that it represents the macromolecular primordium of the rod sacs of the outer segment. Careful study of this morphogenetic material at higher magnification shows that it is formed by short pieces of tubules or vesicles with elements of only 50 to 70 A in diameter having a denser edge and a less dense core.

The cross-section of the cilium shows the nine peripheral filaments and the rest of the matrix occupied by the morphogenetic material (Fig. 2). Both Figs. 1 and 2 suggest that the filaments of the cilium are in direct connection with the above mentioned tubular and vesicular elements of the morphogenetic material.

II. Enlargement of the Primitive Cilium and the Formation of the Primitive Rod Sacs

This second stage which corresponds to the rapid building up of the lamellar material of the rod sacs is illustrated in a progressive way in Figs. 4, 6, 5, and 7. In Fig. 4 the basal part of the cilium remains undifferentiated and shows several ciliary filaments in longitudinal section. One of them is clearly shown to be made of a pair of intertwining filaments of about 140 A in diameter each. These filaments project into the distal portion of the cilium which is enlarged in a mushroom fashion. This region is filled with the morphogenetic material which to a large extent seems to be attached to the filaments of the cilium. On the left side one sees the profiles of several primitive rod sacs, the smallest of which, of about 400 $\times$ 140 A, seem to be in connection with pieces of the ciliary filaments. The formation and progressive enlargement of these primitive rod sacs are difficult to interpret on the basis of macromolecular mechanisms. However, the observation of this and other electron micrographs suggests that they may be the result of the activity of the primitive morphogenetic material found in the matrix of the cilium and may be connected in some way to the ciliary filaments.

In Figs. 6 and 5 one follows the enormous enlargement undergone by the cilium because of the increasing number of primitive rod sacs. Fig. 6 shows that even at this early stage the morphogenesis is asymmetrical, the left side remaining undifferentiated and the bulging right side becoming filled with rod sacs. In Fig. 5 the greatly enlarged tip of the cilium still contains some fine morphogenetic material but numerous and essentially unoriented flattened profiles of tubes and sacs are filling up the entire space.

In Fig. 7 the external segment has become greatly enlarged and has now a cylindrical shape. The entire space surrounded by the surface membrane is now filled with profiles of sacs of different sizes arranged in a parallel fashion. The orientation of most of these sacs does not correspond to that found in the adult since they are longitudinal with respect to the axis of the segment. A few of them, however, are lying transversely as they do in the adult. In this figure the disappearance of the morphogenetic material can also be noted. In Fig. 7 it is also possible to observe the section of an internal segment filled with numerous dense particles and the cross-section of a connecting cilium (*CC*) which is similar to that of the adult retina.

III. Rearrangement of the Rod Sac and Growth of the Adult Rod Cell

The third stage in the morphogenesis of the rod cell is characterized by the remodelling and reorientation of the lamellar material of the external segment. The rod sacs have progressively flattened and the cavity which was very conspicuous, has become practically non-existent by the juxtaposition of the limiting membranes of the sac. At the same time the sacs become oriented transversely to the axis of the segment. This orientation, which will be thereafter permanent, starts in the middle portion of the external segment (Fig. 3). At the same time in the extreme tip and in the lower portion, near the connecting cilium, the rod sacs are still oriented parallel to the axis or disoriented. The observation of numerous rod cells at this stage suggests that both extremities of the outer rod segment may continue to have morphogenetic activity and that this process will determine the growth of this portion of the rod cell.

Observations on the Morphogenesis of the Inner Segment

After the first phase in which the primordium of the inner segment appears as a bulge of cytoplasm containing numerous unoriented mitochondria, a large number of dense particles, a typical basal body, and a small Golgi zone (Fig. 4), this region of the rod becomes elongated and the different components tend to assume the distribution found in the adult (1).

Fig. 8 illustrates the region of the developing inner segments at 12 days of age. The mitochondria with definite double membranes and numerous mitochondrial cristae are now oriented parallel to the axis of the rod and tend to be segregated into the distal zone of the segment corresponding to the ellipsoid. The packing of the mitochondria is, however, not as clear cut as in the adult rod (1, 2). In the rest of the segment a typical large Golgi zone is now apparent and there are also large vesicles and canaliculi surrounded by dense particles which are identified as portions of the endoplasmic reticulum (see reference 1). It is an interesting fact that in earlier stages of development, the vacuoles of the endoplasmic reticulum could not be found while there were numerous dense particles. At 12 days there are some indications of the appearance of a filamentous component in the matrix but typical neuroprotofibrils as in the adult are still not observed.

DISCUSSION

The Primitive Cilium and The Outer Rod Segment

The suggestion concerning the ciliary nature of the connecting cilium and of the outer segment, now proved by the use of the electron microscope (1), is not new in the literature and it was advanced as a hypothesis by several authors based on studies with the optical microscope.

Thus Fürst (5) in the salmon embryo and then Kolmer (6), Held (7), and Retzius (8) in frog, human, and the selachian rods had observed a filamentous structure starting from one or two bodies (diplosomes) extending between the inner and the outer segments.

Following the histogenesis of the retina in the cat (9), human being (10, 11), and in the chicken embryo (12), a filament originating from a centrosome has been observed as the first indication of the external segment. According to Leplat (12) this filament became surrounded by a sheath of mitochondrial nature to constitute the external segment. Description of this fibrous component has also been given by other authors (13–15).

The differentiation of the photoreceptors in man has been thoroughly described by Mann (16). She states that the outer limbs of the rods and cones "would seem to arise by increase in size and reduction in number of pre-existing cilia." . . . "One is therefore safe in drawing at least a close parallel if not a definite derivative relationship between cilia and the primitive outer limbs of the visual elements."

However, this interpretation has not been accepted by other authors. In his review on the retina, Arey (17) states: "From time to time various authorities have referred to the outer segment of the rod and cone, or even to the entire element, as a cuticular or secreted substance, and, this conception has recently been revived by Levi (18)" . . . "Their delicate composition, extreme sensitiveness and specialized structure, including problematical fibrillae apparently negate this view. An interpretation of the rod and cone as robust cilia, homologous with those of the central canal of the embryonic neural tube (Krause) likewise does not appeal."

The observations on the submicroscopic morphogenesis of the rod cell reported here clearly demonstrate that the outer segment is the result of the differentiation of a primitive cilium. In the distal portion of this cilium the complex lamellar system of the rod sacs is formed while a short piece of the proximal or basal part of it remains undifferentiated and constitutes the *connecting cilium* of the adult rod cell (1).

Development of the Rod Sacs and Macromolecular Mechanism of Lamellar Growth

The submicroscopic morphogenesis of the outer segment of the rod consists essentially in the development of numerous flattened rod sacs which fill the apical portion of the primitive cilium and finally constitute the multilayered structure which is characteristic of the adult rod (2, 1).

The intimate macromolecular mechanism which initiates the formation of the primitive rod sacs and determines their lamellar growth is difficult to ascertain with certainty, in spite of the high resolution electron microscopy used in this work. It seems evident, however, that the earliest indication of this process is represented by the so called *morphogenetic material* which is composed of very fine vesicular and tubular structures scattered throughout the matrix of the primitive cilium. This material bears apparently some relationship to the filaments of the cilium and this fact is quite evident in the small primitive rod sacs. In favor of this hypothesis on the origin of the primitive rod sacs by fusion of this vesicular material is the fact that, with the progress in the formation of rod sacs, the morphogenetic material diminishes and tends to disappear or remains only in limited areas in which the differentiation of rod sacs continues.

It is interesting to correlate these findings on the morphogenesis of the retinal rods with the development of the lamellar structure in chloroplasts as recently described by Hodge *et al.* (19), because in the two cases one deals with multilayered lipoprotein structures involved in photochemical reactions. According to these authors "chloroplast lamellae appear to arise by a process involving the 'fusion' of small vesicular elements to give closed double-membrane structures." The similarity with the process apparently taking place in the early formation of the rod sacs seems to be remarkable. However, in this last case it is difficult to interpret the rapid and extensive surface growth of the rod sacs only by the process of fusion of small vesicles. The fact that in the later development the rod sacs appear as large membranous and flattened closed compartments connected by short tubules to the cilium reminds one of the membranous figures, the so called myelin forms, which can be produced with certain lipides and lipoproteins, and which may be of submicroscopic dimensions (20). However, the resemblance between the lamellar growth of the rod sacs and the mechanism of myelin formation may be only superficial. While myelin figures grow at random and in model experiments are unable to produce a coordinated structure (21), in the case of the rod outer segment the morphogenetic mechanisms involved lead to a submicroscopic organization of extraordinary regularity as indicated not only by electron microscopy (2, 1) but also by polarization microscopy (22) and x-ray diffraction (23). The importance of such a repeating macromolecular structure in explaining the chemical and physiological phenomena of photoreception is recently emphasized by Wald (24).

SUMMARY

The morphogenesis of the retinal rods has been studied with the electron microscope in white mice from birth up to the 16th day of age. Observations have been mainly concentrated on specimens of the 8th and 12th days and on the differentiation of the inner and outer segments of the retinal rods. In the morphogenesis of the outer segment three main stages have been considered.

The first stage consists in the development of a primitive cilium projecting from a bulge of protoplasm which constitutes the primordium of the inner segment. A basal body, nine pairs of peripheral filaments, a surface membrane, and a matrix filled with a fine vesicular material have been recognized as components of the primitive cilium. The vesicles are called "morphogenetic material" because it is believed that they represent the macromolecular primordium of the rod sacs of the future outer segment.

The second stage corresponds to the great enlargment of the apical region of the primitive cilium due to the rapid building up of the lamellar material of the rod sacs. The primitive rod sacs appear to be connected with the ciliary filaments. The basal portion of the primitive cilium remains undifferentiated and constitutes the connecting cilium of the adult rod (1).

The third stage consists in the remodelling and reorientation of the rod sacs into their permanent transverse disposition. This process starts in the middle portion of the outer segments and proceeds towards both extremities which can be considered as zones of growth of the outer segment.

The inner segment is at the beginning a bulge of protoplasm containing unoriented mitochondria, a basal body, a small Golgi zone, and numerous dense particles. Then this region becomes elongated and the different components assume the stratified disposition found in the adult (1).

The demonstration that the entire outer segment of the rod cell is the result of the differentiation of a primitive cilium is discussed in view of the conflicting interpretations found in the literature.

The possible macromolecular mechanisms that may be involved in the submicroscopic morphogenesis of the rod sacs are discussed and the possible role of the morphogenetic material is considered.

The results described in this paper confirm and extend the interpretation of the submicroscopic morphology of the adult rod cell as presented in a previous paper (1).

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EXPLANATION OF PLATES

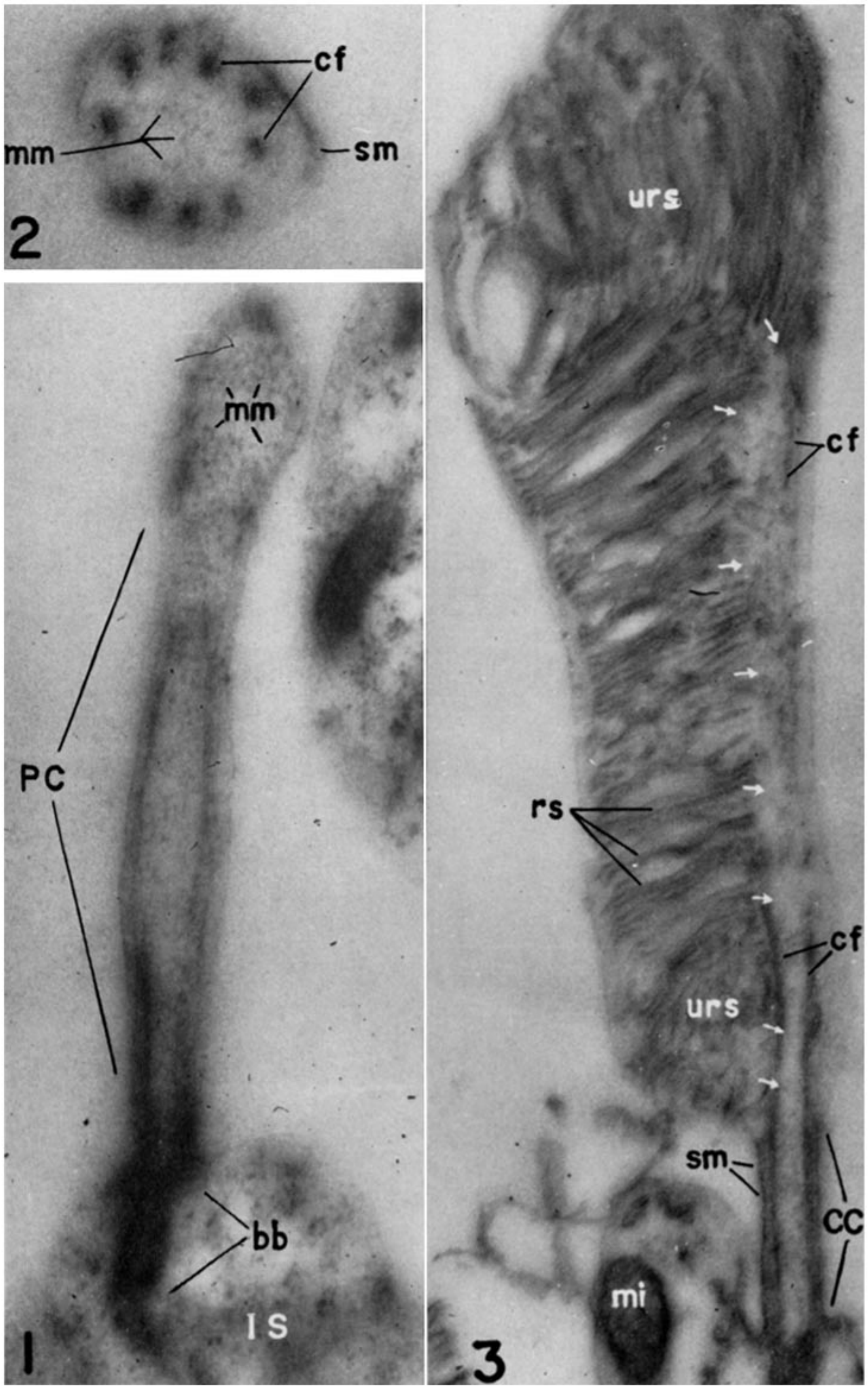
bb, basal body.
CC, connecting cilium.
cf, ciliary filaments.
dp, dense particles.
er, endoplasmic reticulum.
Gs, Golgi apparatus.
IS, inner segment.
mi, mitochondria.
mm, morphogenetic material.
OS, outer segment.
PC, primitive cilium.
prs, primitive rod sacs.
RN, rod nucleus.
rs, rod sacs.
sm, surface membrane.
urs, unoriented rod sacs.

PLATE 70

FIG. 1. Electron micrograph of the most distal portion of a rod cell of the retina of an 8-day-old white mouse. Longitudinal section showing at the bottom a portion of a primordium of the inner segment (*IS*) with an asymmetrical basal body (*bb*) which is in continuity with the primitive cilium (*PC*). In the cilium note the surface membrane, the ciliary filaments, and the fine vesicles, the so called morphogenetic material (*mm*), which fills the matrix of the cilium and concentrates in its apical portion. × 54,000.

FIG. 2. Micrograph of a cross-section of the apical portion of a primitive cilium showing the surface membrane (*sm*), the nine ciliary filaments (*cf*), and the morphogenetic material (*mm*). × 70,500.

FIG. 3. Micrograph of a longitudinal section of the outer segment of a rod cell of a 12-day-old white mouse. The morphogenesis of the outer segment is almost completed and the largest portion of it shows the transversely arranged rod sacs (*rs*) typical of the adult rod. In both extremities there is a region of unoriented rod sacs (*urs*) which are probably still engaged in the process of morphogenesis and growth. Marked with white arrows there is a zone which corresponds exactly in size and constitution to the primitive cilium. In this region the ciliary filaments (*cf*) and some morphogenetic material are observed. The basal portion of the cilium has differentiated into the connecting cilium (*CC*) of the adult rod. × 52,000.

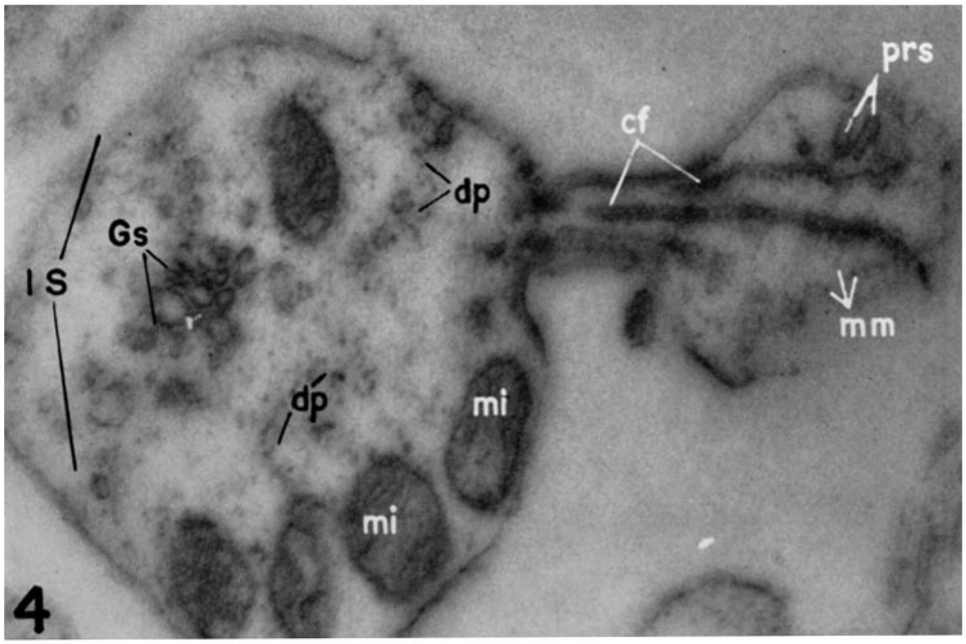


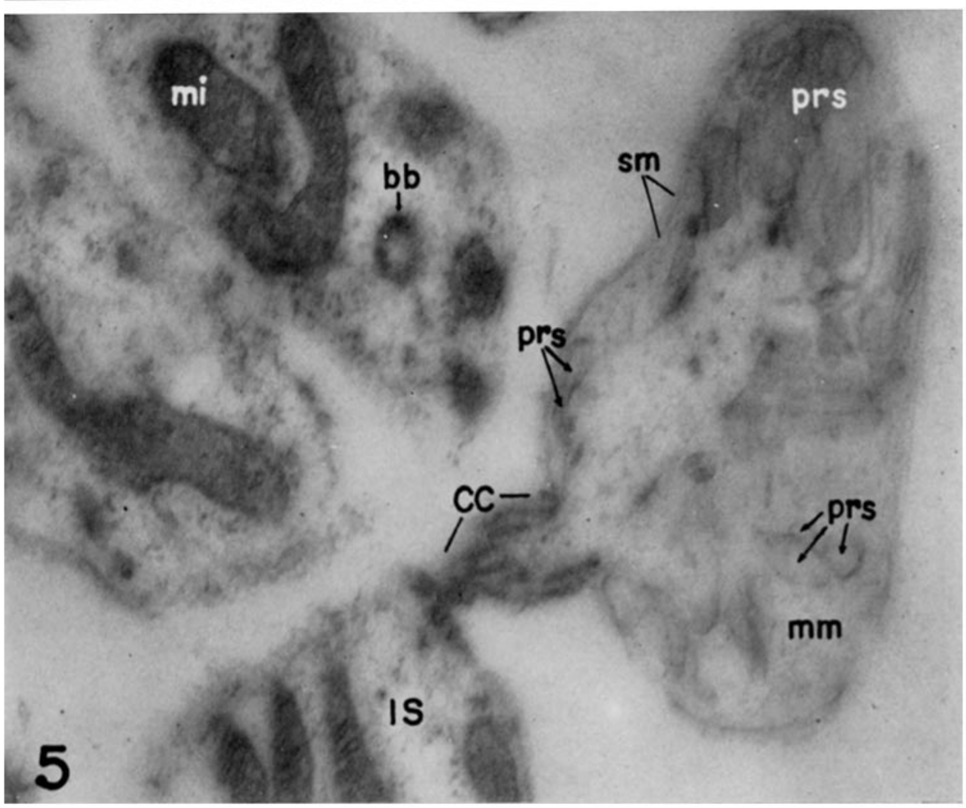
(De Robertis: Morphogenesis of retinal rods)

PLATE 71

FIG. 4. Electron micrograph of a differentiating rod cell of an 8-day-old white mouse. To the left is the round profile of the primordium of the inner segment containing mitochondria (*mi*), dense particles (*dp*), and Golgi material (*Gs*). To the right is the primitive cilium, enlarging in its apical portion and showing the double ciliary filaments (*cf*), the morphogenetic material (*mm*), and the most primitive and smallest rod sacs (*prs*) (see description in the text). × 70,500.

FIG. 5. Shows a stage similar to that in Fig. 4 but with a greater enlargement of the apical portion of the primitive cilium. Within the bulging zone numerous primitive unoriented rod sacs (*prs*) are differentiating but there are still spaces containing morphogenetic material. The connecting cilium (*CC*) is clearly observed. × 55,500.



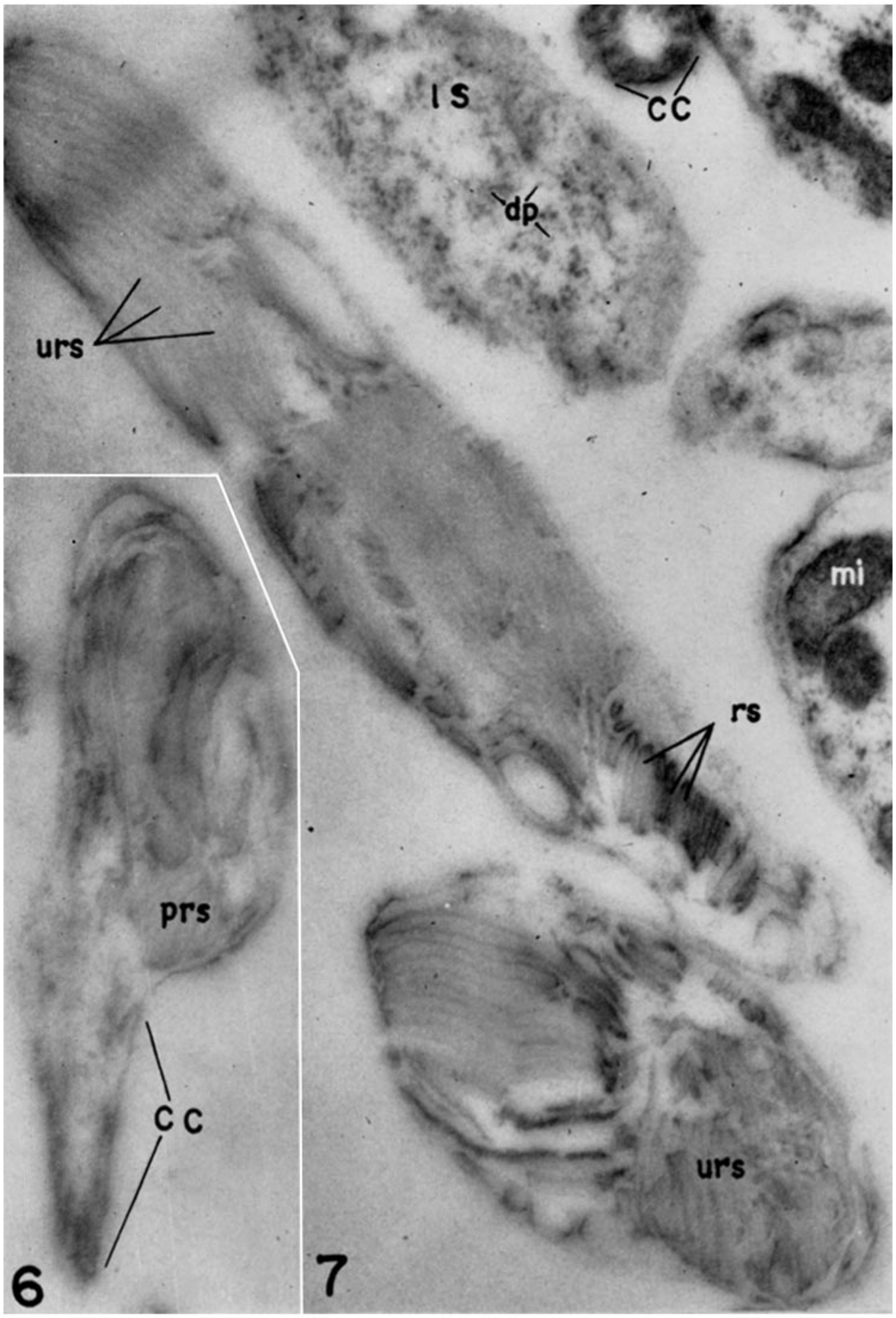


(De Robertis: Morphogenesis of retinal rods)

PLATE 72

FIG. 6. Description similar to that for Fig. 5. Micrograph shows that the morphogenesis of the outer segment is already asymmetrical, the right bulging side containing the primitive rod sacs. The connecting cilium is observed at (*CC*). × 54,000.

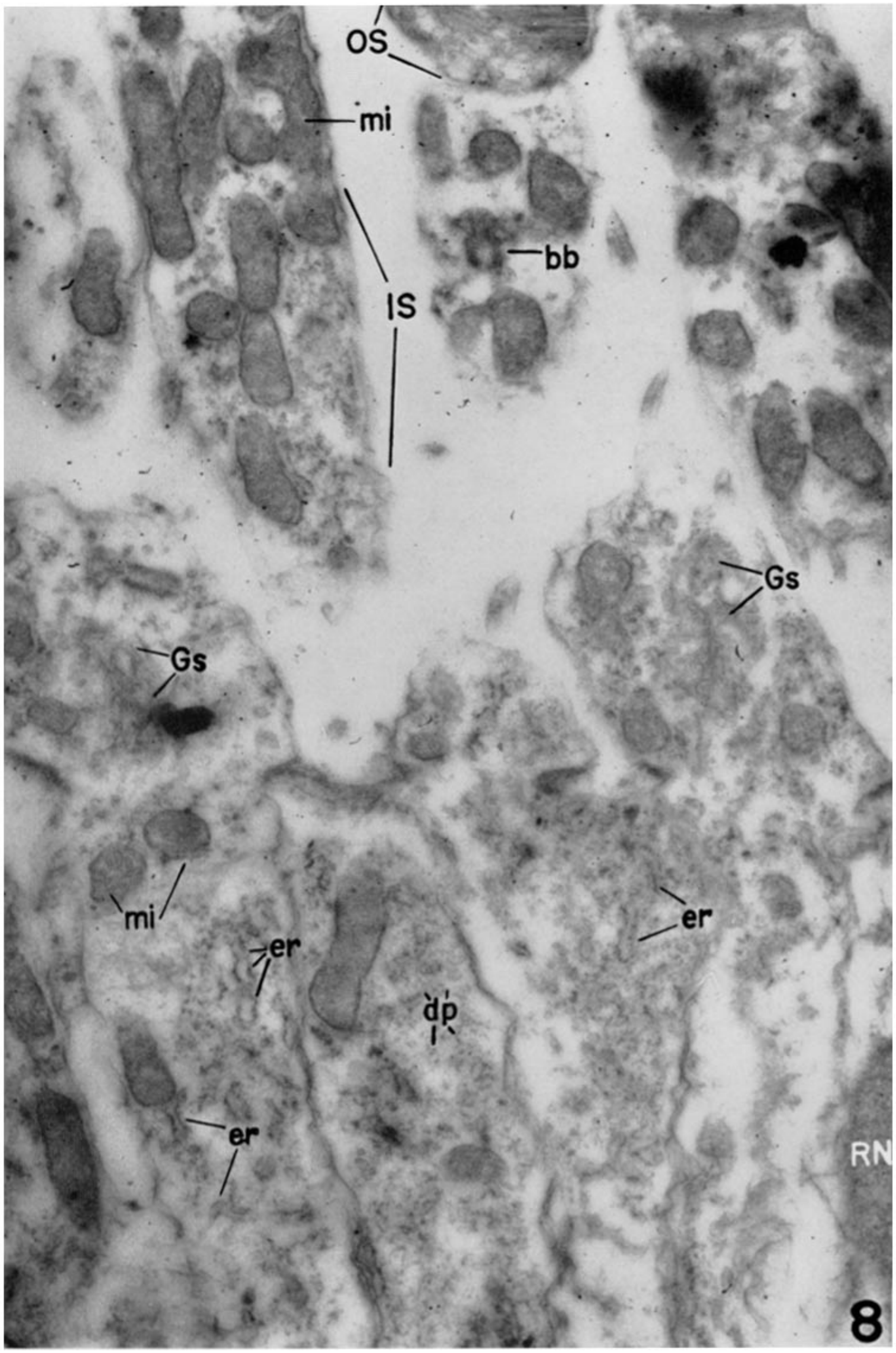
FIG. 7. Electron micrograph of developing rod cells in an 8-day-old mouse. The large cylindrical outer segment is filled with unoriented rod sacs (see description in the text). Note the cross-section of a connecting cilium (*CC*) and the abundance of dense particles (*dp*) in the inner segment (*IS*). × 55,500.



(De Robertis: Morphogenesis of retinal rods)

PLATE 73

FIG. 8. Low power electron micrograph of a zone of the retina of a 12-day-old white mouse containing developing inner segments (see the description in the text). × 27,500.



(De Robertis: Morphogenesis of retinal rods)