Some Observations on the Ultrastructure and Morphogenesis of Photoreceptors

E. DE ROBERTIS

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

The photoreceptors of the vertebrate retina are highly differentiated cells having a dual function. They act as receptors of light at the outer segment and as transmitters of the resulting message at their innermost end.

Morphologically they can be considered as nerve cells having a high degree of submicroscopic organization and specially differentiated portions. Thus, at the distal segment there is a system of lipoprotein membranes involved in photochemical mechanisms and at the proximal end a special synaptic junction whose fine structure changes in different physiological conditions (De Robertis, 1958).

Another characteristic of the visual cells is the separation of the cytoplasm into definite segments. Being very elongated cells many micra long, they show a compartmental arrangement along the axis which refers not only to its fine structure, but also to its chemical organization. Thus the visual pigments are exclusively localized in the outer segment and the enzymes of the oxidative phosphorylation and the Krebs cycle are mainly distributed in the distal part of the inner segment where mitochondria are concentrated at the so called ellipsoid (Text-fig. 1).

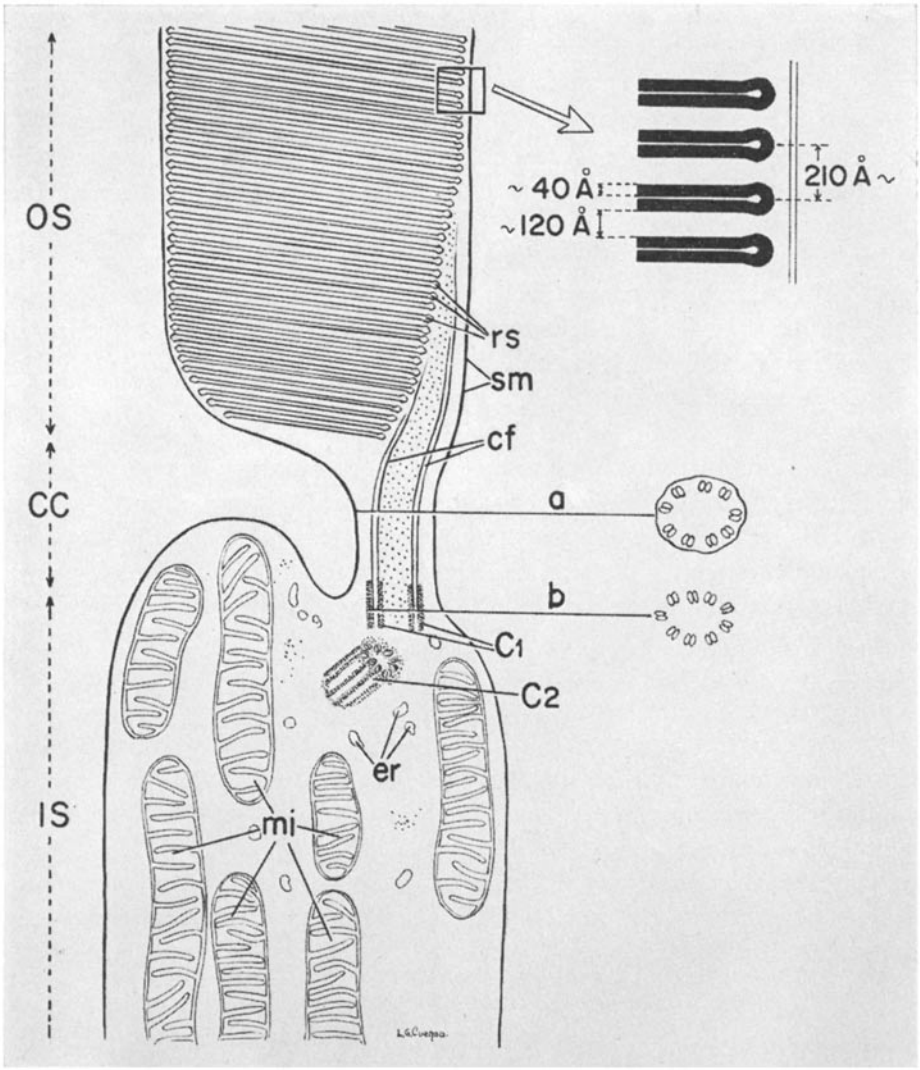
The studies of Lowry *et al.* (1956) and Schimke (1957) have shown that the inner segment is rich in enzymes of the respiratory system while the most proximal parts of the cell are abundant in glycolytic enzymes. Noell (1958) has recently emphasized the importance of this special enzymatic distribution in relation to the action of certain drugs that destroy photoreceptors. Recently the action of iodoacetate on the fine structure of photoreceptors has been studied (Lasansky and De Robertis, 1959).

Ultrastructure of Visual Cells The submicroscopic organization of photoreceptors was first studied with polarization microscopy (Schmidt, 1937) and more recently with the electron microscope. The use of the high resolving power of this instrument has been decisive in elucidating the morphological details of structure. In 1949 Sjöstrand recognized the existence of a layered

This paper is supported by Grant B 1549 of the National Institutes of Health, United States Public Health Service.

structure of membranes in rod outer segments disintegrated sonically and studied under the electron microscope. In 1953 the existence of this fine structure was confirmed in thin sections and Sjöstrand described the outer segment as composed of a pile of double membranes or discs.

In 1956 we described some additional features which are represented in



TEXT-FIGURE 1. Diagram based on electron microscope observations of rod cells of mammals. At the left are indicated the outer segment (*OS*), the connecting cilium (*CC*), and the inner segment (*IS*). At the right cross-sections through the connecting cilium (*a*) and the centriole (*b*) C_1. C_1 and C_2, the two centrioles; *rs*, rod sacs; *Cf*, ciliary filaments; *sm*, surface membrane; *mi*, mitochondria; *er*, endoplasmic reticulum.

Text-fig. 1. The outer segment was found to consist of a stalk of flattened sacs or cisternae limited by a membrane. Furthermore the bundle of filaments connecting the outer and inner segments was recognized to be of ciliary nature and described as the *connecting cilium* (De Robertis, 1956*a*). Other details of fine structure were described in the inner segment particularly the concentration of mitochondria at the distal end (ellipsoid), together with the presence of a basal body and of endoplasmic reticulum. In the proximal portion of this segment a Golgi complex and neuroprotofibrils of 160 to 200 A were also recognized.

Fig. 1 is an electron micrograph of a rod cell of the rabbit showing the fine structure of the outer segment and the connecting cilium. At the bottom a basal body is shown that has the typical structure of a centriole and whose fibrillar components are in continuity with the ciliary fibrils. Unpublished observations on this structure show that there are two centrioles in this region of which only one is in continuity with the cilium (Fig. 3, *C1*). The other centriole (*C2*) lies deeper in the segment and is oriented forming a 90° angle with *C1*. Cross-sectional and longitudinal views of this centriole *C2* are shown in Figs. 3 and 4. The similarity with the centrioles of other cells described by De Haven and Bernhard (1956) and others is evident.

The outer segment of the rod is composed of flattened sacs made of membranes 40 Å thick and separated by spaces of 110 to 120 Å between the sacs. At the lateral ends of the sacs there is a typical buttonhole enlargement below the surface membrane (Text-fig. 1 and Fig. 1).

Fine Structure of the Cone Cell While there is at present an extensive literature on the rods, very little has been published on the fine structure of the cones (for literature see De Robertis and Lasansky, 1958).

Recently with Lasansky we have studied under the electron microscope the retinal cones of the rabbit. These observations have led us to postulate that although there are important morphological differences between rods and cones there is a single plan of submicroscopic organization. In the cone cell of Fig. 2 the outer segment is formed also of flattened double membrane sacs, but at the proximal region the sacs are not as well oriented and some of them may be replaced by clear vesicles of varying dimensions. In this figure the connecting cilium of the cone is visible and also a part of the very large inner segment containing the mitochondria of the ellipsoid.

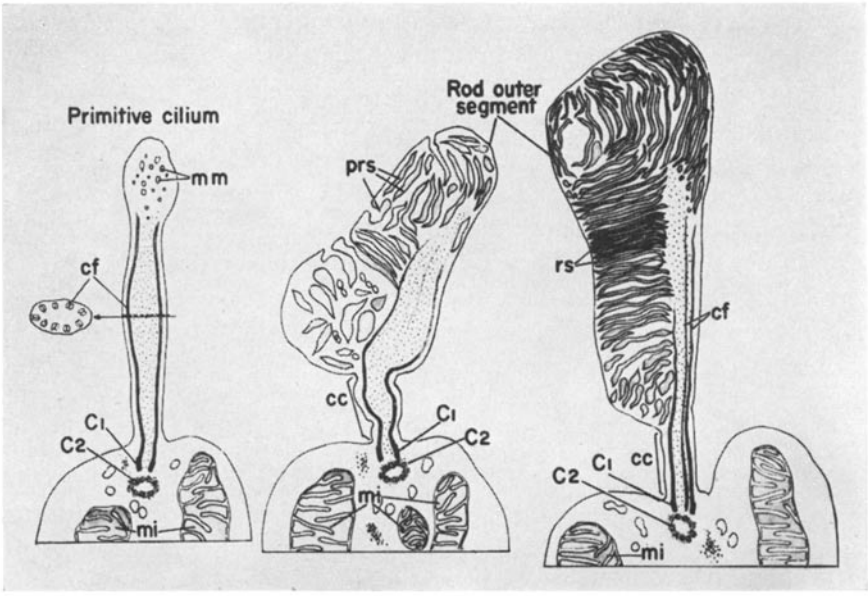
The similarity in the general pattern of organization agrees with the view that the basic chemical mechanisms of the cone and rod vision are essentially similar (Wald, 1955). It also confirms the close relationship existing between structure and function in photoreceptors. Furthermore the fact that the cone has a connecting cilium similar to that of the rod, may be indicative of a similar morphogenetic origin (De Robertis, 1956*b*).

Beyond this structural similarity of organization and morphogenesis

between rods and cones there are notable differences in fine structure which may probably be related to their specific function and chemical organizations.

MORPHOGENESIS OF PHOTORECEPTORS

Following the demonstration of the ciliary nature of the fiber connecting the rod outer and inner segments the differentiation of the photoreceptors during morphogenesis was studied (De Robertis, 1956*b*).



TEXT-FIGURE 2. Diagram indicating different stages in the development of the rod outer segment.

In retina of rats and mice observed between birth and the 18th day of age, the different stages of the development of the outer segment could be followed and three main stages were recognized. In the first stage a primitive cilium projects from a bulge of cytoplasm which contains mitochondria and RNP granules (the primordium of the inner segment).

This cilium contains the 9 pairs of filaments characteristic of cilia (Fawcett and Porter, 1954) and the two basal centrioles described above (Fig. 3). The apical end of the primitive cilium is filled with a vesicular (or tubular) material which was called "morphogenetic material," because it was thought to be related to the formation of the rod sacs of the outer segment.

The second stage consists in the great enlargement of the apical region of the primitive cilium due to the rapid building up of vesicles and cisternae

that constitute the primitive rod sacs (Fig. 4). At the same time the innermost region of the cilium remains unaltered constituting the connecting cilium of the adult rod. This process of membrane synthesis seems to be related to the filaments of the cilium, which appear to have a tubular structure, and to the morphogenetic material discussed above.

However, new observations tend to indicate that the outer membrane of the cilium, probably under the influence of the ciliary filament (induction?) may invaginate and form the primitive rod sacs. This interpretation has been recently postulated by Tokuyasu and Yamada (1959). The careful examination of the developing outer segment of Fig. 4 shows several continuities between the outer membrane and the primitive sacs being formed. It is interesting that this effect is only present on one side of the cilium which makes the development asymmetrical. The primitive cilium remains unchanged on one side of the outer segment while in the other the sacs develop in great profusion (Fig. 4 and Text-fig. 2).

The third stage consists in the remodelling and reorientation of the sacs into their permanent transverse disposition. This process starts in the middle portion of the outer segment and proceeds toward both extremities. These two regions maintain for some time an irregular disposition of the sacs and can be considered as zones of growth of the outer segment (Text-fig. 2).

SUMMARY

Some new details of fine structure of the rod and cone cells of mammals are described in the adult and during the process of development and differentiation of photoreceptors.

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PLATE 1

FIGURE 1. Electron micrograph of the rod cell of the adult rabbit. *ROS*, rod outer segment with the rod sacs (*rs*) and the surface membrane (*sm*). *RCC* (rod connecting cilium) with the ciliary filaments (*cf*). C_1, centriole connected with the connecting cilium. × 95,000.

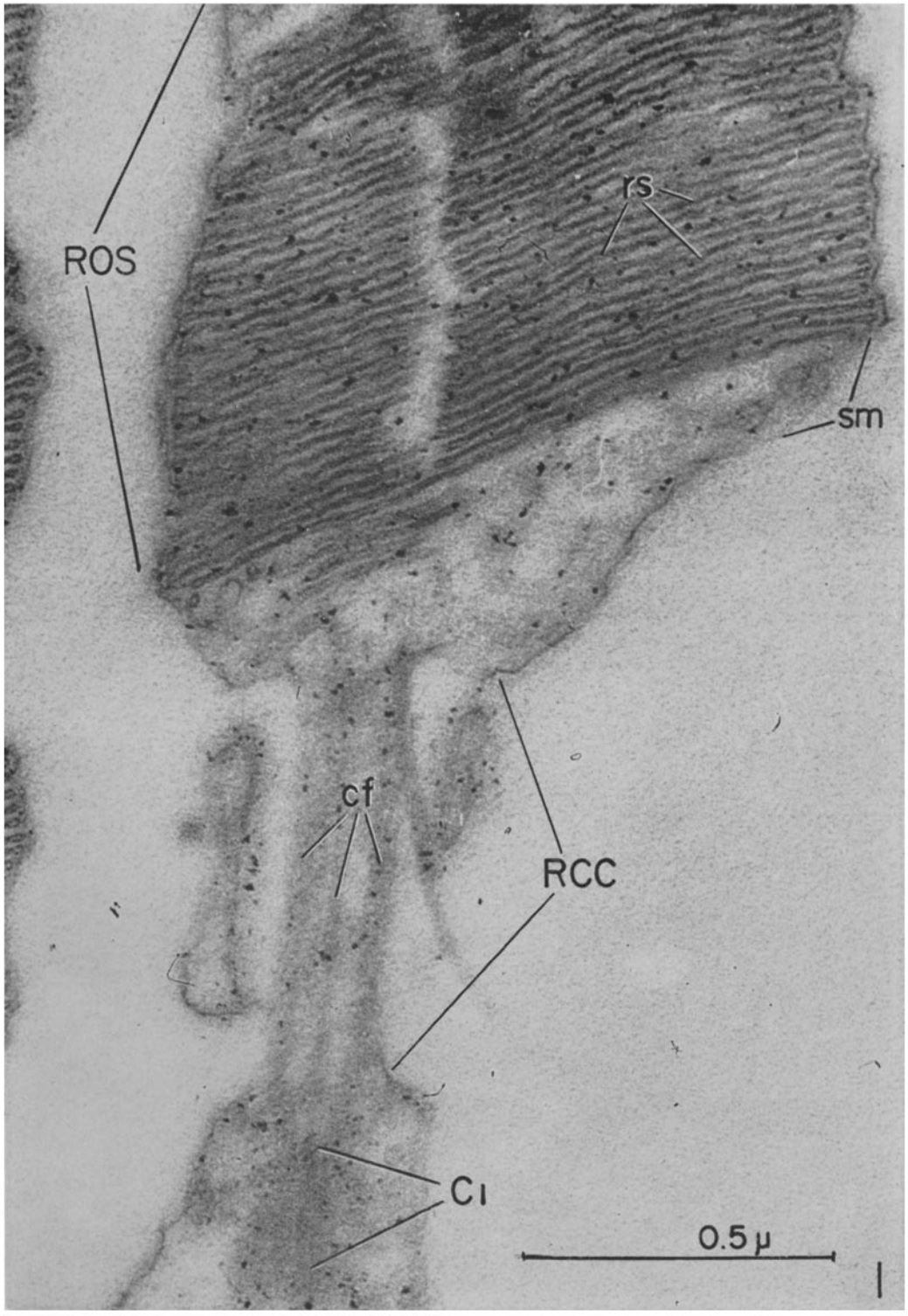

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PLATE 2

FIGURE 2. Electron micrograph of the cone cell of the adult rabbit. *COS*, cone outer segment, with the cone sacs (*cs*) and a region of unoriented cone sacs (*ucs*), *ccc*, cone-connecting cilium, with the ciliary filament (*cf*); *CIS*, cone inner segment, with mitochondria (*mi*). × 48,000. De Robertis and Lasansky (1958).

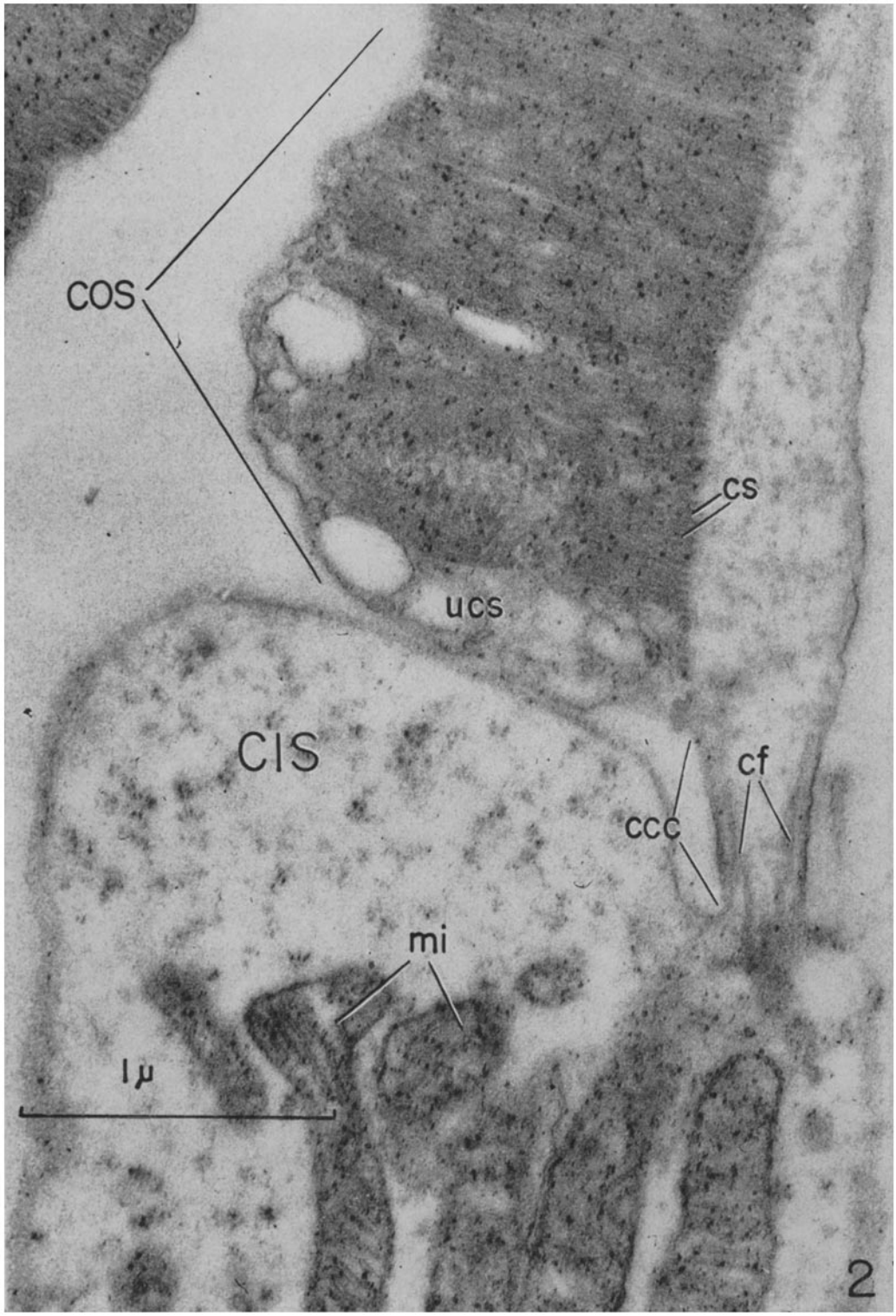

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PLATE 3

FIGURE 3. Electron micrograph of a developing photoreceptor. *PC*, primitive cilium arising from the primordium of the inner segment (*IS*). *cf*, ciliary filaments; *mm*, morphogenetic material; *sm*, surface membrane. C_1 and C_2, the two centrioles with an orientation at right angle; *mi*, mitochondria. × 80,000.

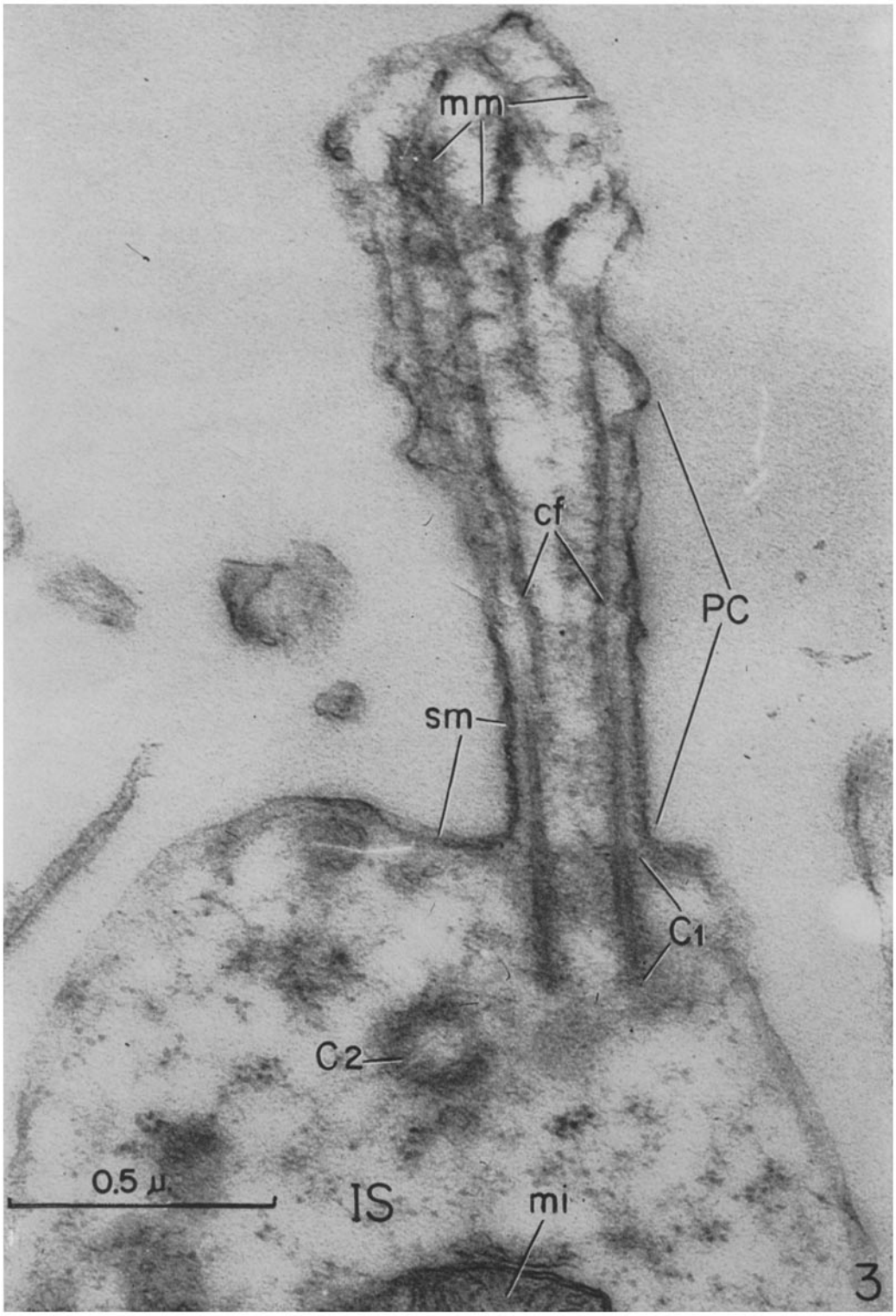

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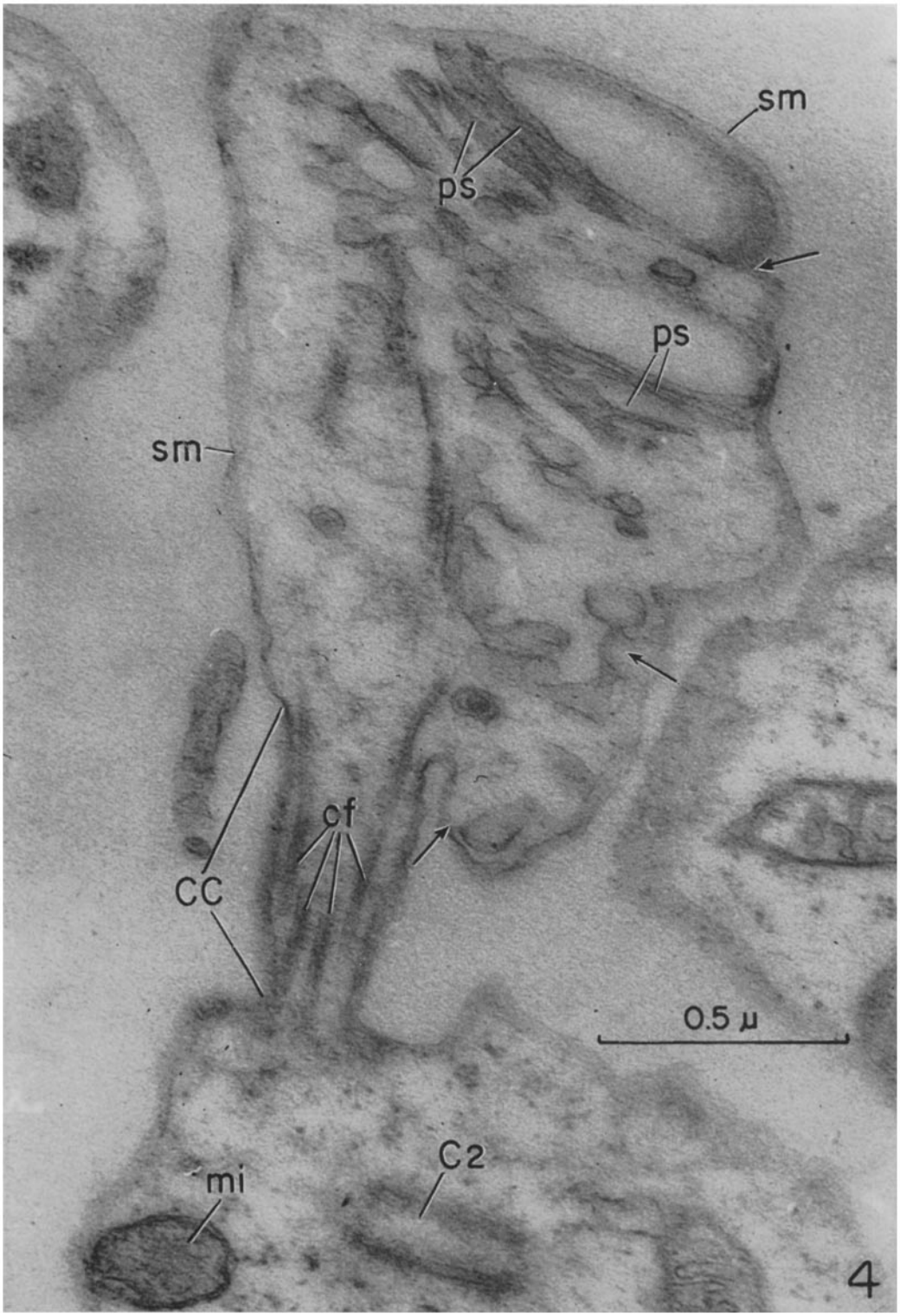
PLATE 4

FIGURE 4. Electron micrograph of a further stage in the development of the photoreceptor. The primitive cilium of Fig. 4 has greatly enlarged in the upper end. This is filled with membranes forming primitive sacs (*ps*). With arrows are indicated some of the continuities of the primitive sacs with the surface membrane (*sm*). See that all primitive sacs are being formed on the right side of the cilium. The lower portion of the primitive cilium is now transformed into the connecting cilium (*CC*); C_2, centriole; *mi*, mitochondria; *cf*, ciliary filaments. × 76,000.



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