The Fine Structure of the Retina Studied with the Electron Microscope

IV. Morphogenesis of Outer Segments of Retinal Rods

By KIYOTERU TOKUYASU,* Ph.D., and EICHI YAMADA, M.D.

(*From the Department of Anatomy, School of Medicine, Kurume University, Kurume, Japan*)

PLATES 121 TO 124

(Received for publication, August 29, 1958)

ABSTRACT

The morphogenesis of the outer segments of retinal rods was studied mainly in the kitten before the opening of the eye, and the probable sequence of the morphogenetic stages is deduced. Since the development of retinal rods is not synchronous, the deductions were based on observations of many single and serial sections.

One centriole extends ciliary tubules of about 0.5 μ long, in the growing primitive cilium. Beyond this length, each ciliary tubule becomes a row of small vesicles (called "ciliary vesicles" in this paper), which penetrate into the distal region of the cilium.

Where the ciliary vesicles establish contact with the plasma membrane of the distal region of the cilium, more or less deep infoldings of the plasma membrane are observed. In the distal region can be seen rows of tubular or vesicular structures. A few of these membranous structures are continuous with the bottoms of the infoldings.

At the following stage, the infoldings disappear and the ciliary vesicles lose contact with the distal plasma membrane. Nonetheless, the formation of the tubular structures continues in the distal region of the primitive outer segment.

The tubular structures appear to be transformed into the primitive rod sacs by sidewise enlargement. At a subsequent time, presumably, these primitive rod sacs flatten and are rearranged into a position perpendicular to the long axis of the outer segment.

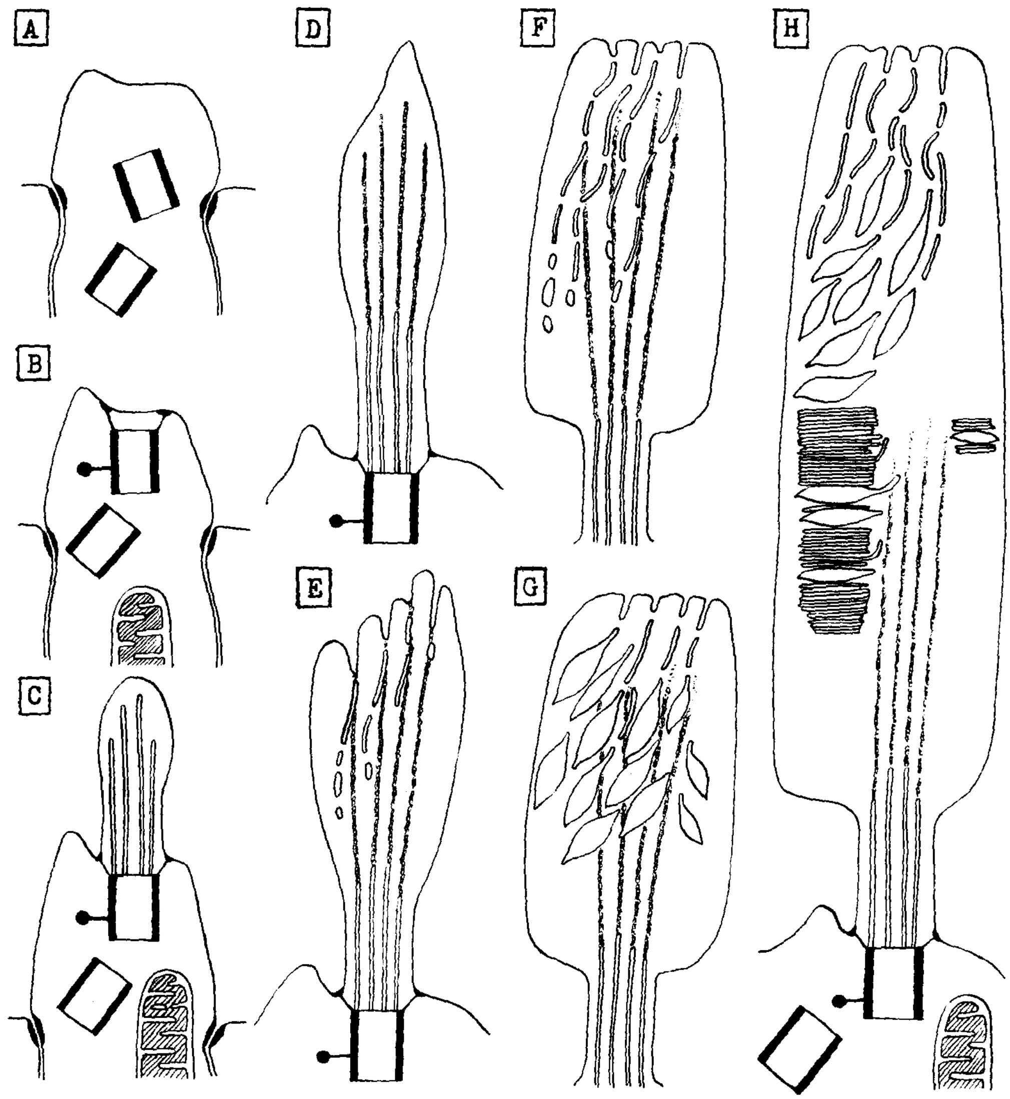
The detailed structure of the basal body of the connecting cilium was also studied by means of serial sections.

Electron microscope studies of retinal rods of vertebrates (1–4) have revealed the presence of numerous flattened sacs or cisternae piled in the outer segments of the rods, and have shown a connecting cilium (2) between the outer and inner segments.

De Robertis (5) has studied the developmental process of the rod with the electron microscope and observed that primitive rod sacs appeared first rather randomly distributed in the distal region of the primitive cilium. He has also noted that the rapid building of rod sacs was followed by the great enlargement of the distal region and by the reorientation of sacs into their permanent position perpendicular to the long axis of the rod. He assumed that the primitive rod sacs were formed by fusion of vesicular material, the so called "morphogenetic material."

At the same time, however, he stated: "... 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 present authors encountered the same difficulty and consequently tried to reexamine in detail the morphogenesis of the retinal rod.

* Present address: Section of Scientific Instruments, Taga Works, Hitachi Products, Ltd., Hitachi-shi, Ibaragi-ken, Japan.



TEXT-FIG. 1. Schematic representation of the probable sequence of morphogenesis of the outer segment of a retinal rod.

The distal portion of a visual cell protrudes through the outer limiting membrane of the retina and two centrioles appear in the protrusion (*A*). One of them attaches to the plasma membrane of the distal region of the protrusion with filamentous stalks and extends cudgel-shaped projections out of its trunk. It is destined to be the basal body of the primitive cilium (*B*). The basal body extends ciliary tubules and the primitive cilium is formed (*C*). The distal region of the cilium enlarges and when the cilium grows beyond the length of about 0.5 μ, each ciliary tubule becomes a row of small vesicles (ciliary vesicles) (*D*). The distal region enlarges more. Infoldings of the plasma membrane appear where the ciliary vesicles establish contact with the plasma membrane in the distal region. At the bottoms of the infoldings, rows of membranous structures, mostly of tubular nature, are found (*E*). The width of the distal region reaches almost that of the mature outer segment and the region as a whole shows the short primitive shape of the outer segment. The basal part of the cilium, about 0.5 μ, remains largely unaltered and becomes the connecting cilium. The distal plasma membrane of the outer segment does not appear to have the infoldings but formation of tubular structures proceeds actively (*F*). The tubular structures enlarge laterally and transform into primitive rod sacs (*G*). The primitive rod sacs flatten and acquire the final disposition normal to the long axis of the outer segment. The formation of tubular structures or their transformation into primitive rod sacs continues in the distal region of the outer segment above the mass of the flattened rod sacs, whereas the ciliary vesicles appear to fade out in the central region (*H*). For further details, see the text.

Materials and Methods

Retinal specimens were obtained from kittens 1 to 5 days old and from mice 1 to 10 days old. In all cases the eyes were not yet open. The eye balls were removed, with animals under ether anesthesia, and cut into pieces of appropriate size which, after careful removal of the vitreous, were fixed with the modified Palade's fixative (6) for 0.5 to 1.5 hours at 2°C. After dehydration through increasing concentrations of ethanol and impregnation in three changes of the plastic, the specimens were embedded in butyl methacrylate, which was polymerized at 45°C. for 24 hours. Sections were cut with a Porter-Blum microtome at the setting of 1/40 to 2/40 μ and were examined mostly with a Hitachi HS-5 electron microscope.

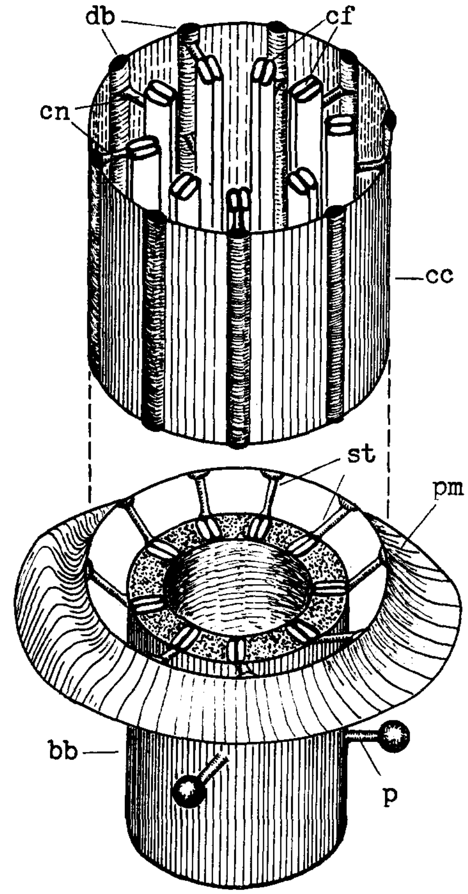
The kitten retina yielded better preparations than mouse retina, presumably because of its larger size, and for this reason, the present description concerns mainly the former material. The preparations of these materials was more difficult than in the case of adult animals, possibly because of the relatively larger amount of water contained in the cells of new born animals.

OBSERVATIONS

The retinal rods do not develop synchronously, at least not in the case of kitten or mouse retina. In the present study, the probable sequence of the morphogenetic events is deduced from the observations of a number of sections of rods. The morphogenetic stages are consecutively numbered and described in the order of their assumed sequence.

1. The first event in the morphogenesis of the retinal rod seems to be the protrusion of the distal portion of the visual cell through the outer limiting membrane of the retina and the appearance of a diplosome in the distal region of the protrusion (Text-fig. 1 *A*). The centriole closer to the distal end of the protrusion extends ciliary tubules,[1] thus forming the primitive cilium (5) (Fig. 1 and Text-fig. 1 *B* and *C*).

2. When the length of the cilium reaches about 1 to 1.5 μ, the distal part of the cilium enlarges gradually and the cilium as a whole shows a somewhat scoop-like profile (Figs. 2 to 4 and Text-fig. 1 *D*). At this stage, the ciliary tubules grow to the length of about 0.5 μ. Beyond this length, each tubule becomes a row of small vesicles (Figs. 3 and 4, and Text-fig. 1 *D*). These vesicles seem to be a derivative of the ciliary tubules because of their mutual continuity, and are therefore called "ciliary vesicles" in this paper.



TEXT-FIG. 2. Schematic representation of structures of the basal body and connecting cilium.

The basal body (*bb*) attaches to the plasma membrane (*pm*) of the inner segment with nine short stalks (*st*) and extends cudgel-shaped projections (*p*) out of its trunk. The plasma membrane of the connecting cilium (*cc*) seems to be dense along each ciliary tubule (*cf*). Occasionally a connection (*cn*) is seen between each ciliary filament and the corresponding dense band (*db*). For further details, see the text.

3. At the next stage, a marked morphological change is observed at the level of the plasma membrane covering the distal end of the cilium. The plasma membrane shows a number of more or less deep infoldings (Figs. 5 to 13) and as a result the cell surface becomes quite irregular or rough. It has a smooth appearance before (Figs. 1 to 4) and after this stage (Figs. 14 to 20). The examination of many serial sections of rods at this stage reveals that almost all of these infoldings are found where the rows of ciliary vesicles establish contact with

[1] Since the structure found within the cilium shows a tubular nature, the term "ciliary tubule" is used throughout this paper instead of "ciliary filament" (11).

the plasma membrane (Figs. 5 to 8 and Text-fig. 1 *E*). In the cytoplasm of the distal region of the cilium at this stage, numerous tubular structures are seen arranged in rows extending from the bottoms of infoldings. Some of the tubular structures are continuous with the bottoms of the infoldings (Figs. 9 to 13 and Text-fig. 1 *E*).

4. The enlargement in diameter of the distal region of the primitive cilium proceeds until its maximum width is approached, about 1 μ, and until the distal region shows the short primitive shape of the outer segment (Figs. 14 to 16 and Text-fig. 1 *F*). The basal part of the cilium, about 0.5 μ long, remains without any significant alteration and takes the shape of the so called "connecting cilium" (2) (Figs. 14 to 16 and Text-fig. 1 *F*).

At this stage, numerous tubules are observed together with a small number of large vesicles in the outer segment and most of these structures are disposed in such a way that they appear to be directed towards the distal end of the outer segment (Figs. 14 to 16 and Text-fig. 1 *F*). In addition, some of the tubules are found to be continuous with the plasma membrane of the outer segment (Fig. 16). It should be emphasized that in the material of this study, contrary to the description of De Robertis (5), the so called "morphogenetic material" is not found in abundance during these stages.

The ciliary vesicles become rare in the distal region of the outer segment and the rows of the ciliary vesicles seem to lose gradually their connections with the distal plasma membrane, concomitant with the growth of the distal region.

5. At the following stage, only a few tubules can be observed, whereas large vesicles become predominant in the distal region of the outer segment (Figs. 17 and 18, and Text-fig. 1 *G*). These vesicles seem to correspond to the so called "primitive rod sacs," described by De Robertis (5). Profiles of the primitive rod sacs show somewhat expanded oval shapes often with sharply pointed ends. The long axes of most of these profiles are directed towards the tip of the outer segment like the tubular structures in the prior stage (Figs. 17 and 18, and Text-fig. 1 *G*). These findings suggest that the tubular structures transform into the primitive rod sacs by lateral enlargement. Probably, however, the primitive rod sacs are more sensitive to the changes in fixation medium than are the mature sacs, and their distended appearance may be an artifact.

6. In the outer segment at the next stage, flattened rod sacs together with a small number of distended primitive rod sacs are found piled regularly and disposed more or less normal to the long axis of the outer segment (Figs. 19 and 20, and Text-fig. 1 *H*). The position of the mass of the primitive rod sacs relative to the base of the connecting cilium at the prior stage (Figs. 17 and 18) corresponds approximately to that of the mass of the flattened rod sacs (Figs. 19 and 20). These findings are taken to indicate that the primitive rod sacs are flattened and rearranged into a transverse position relative to the long axis of the outer segment.

The growth in length of the outer segment continues, while the width remains essentially constant (compare Figs. 14 to 18 with Figs. 19 and 20). In the newly elongated region of the outer segment above the mass of flattened rod sacs, numerous tubular structures are found close to the distal plasma membrane, although the ciliary vesicles appear restricted to the central region of the outer segment and do not seem to reach the distal region.

The continuous formation of tubular structures in the distal region of the elongating outer segment is apparently followed by their successive transformation into rod sacs until the fully extended outer segment is filled with regularly piled rod sacs. Some flattened sacs appear to be continuous with tubular structures (Figs. 19 and 20) suggesting that sometimes the terminal portion of a tubular structure remains unchanged, without undergoing enlargement or subsequent incorporation into a rod sac. In fact, such tubular structures continuous with rod sacs are often recognizable even in the outer segment of adult vertebrates (Figs. 21 and 22). This detail as well is suggestive of the transformation of tubular structures into rod sacs.

The examination of the basal body of the connecting cilium by means of serial sections reveals several clavate projections which are attached to the trunk of the basal body by the ends of their tails (Figs. 23, 24 *G* and *H*, and Text-fig. 2) and also nine delicate short stalks connecting the distal periphery of the basal body with the adjacent plasma membrane (Figs. 23, 24 *D*, *E*, and *F*, and Text-fig. 2). The head part of the clavate projections show a diameter of about 60 to 80 mμ and the tail part is about 60 to 100 mμ long and 15 to 20 mμ wide. The delicate short stalks connecting the basal body with the plasma membrane measure about 60 to 100 mμ in length and about 10 to 15 mμ in width.

The plasma membrane of the connecting cilium seems to be denser along each pair of ciliary tubules than in between (Figs. 24 *A* to *C*). Between each pair of ciliary tubules and the corresponding dense band, a connection is sometimes observed (Figs. 24 *A* to *C*, and Text-fig. 2).

In continuity with the bottom of the basal body, a bundle of fine filaments is often observed descending deep into the inner segment. Sometimes a cross-striation with a repeat-unit of about 55 mμ is clearly recognized in the bundle (Figs. 23 and 25). A similar structure has been reported in the case of the guinea pig (7).

DISCUSSION

It is reasonable to assume, on the basis of the above observations, that the tubules and vesicles found in the developing outer segment are the structural precursors of the rod-sacs. Indeed the evidence suggests that these tubules grow into primitive rod-sacs which subsequently mature by flattening and change their orientation to that of normal to the long axis of the outer segment. As far as the source of these membranous tubular structures is concerned, several possibilities can be envisaged. In the early stages of morphogenesis, tubules and vesicles of ciliary origin are found aligned in rows extending from the bottoms of, and sometimes continuous with, the infoldings formed by the distal plasma membrane. At a later stage, when the formation of rod-sacs proceeds rapidly, the distal part of the outer segment is occupied by numerous tubules which are usually disposed parallel to the long axis of rod, and occasionally appear in continuity with the plasma membrane. At this stage ciliary tubules and vesicles seem to be restricted to the proximal 1.5 μ of the outer segment. The findings lead us to consider two possible sources for these initial membranous structures (tubules and vesicles) namely: (1) the plasma membrane of the outer segment; and (2) the ciliary tubules and vesicles. The information at hand suggests that both sources supply membranous material and that the first is probably more important. It is tempting to speculate, on the basis of the morphogenetic sequence described, that contact of the plasma membrane with the growing ciliary structures initiates the formation of repeated invaginations and tubules. According to this hypothesis, which obviously should be tested further, the rod-sacs are essentially derived from the plasma membrane, the ciliary structures providing only a "trigger" for the morphogenetic process. In any case, it should be pointed out that the formation of rod-sacs implies large scale synthesis of proteins and lipids in the primitive retinal rod. The sites of synthesis may not be the same as the sites at which tubules and vesicles are formed. The cell membrane as well as the ciliary tubules may function only as carriers for materials synthesized elsewhere in the cell.

It should be emphasized that all the structures we described in the primitive rods were encountered in retinal specimens collected before the opening of the eyes. The essential morphological features of the mature rod, *e.g.*, the presence of piles of flattened sacs, perpendicular to the long axis of the outer segment, are already established in retinas little affected by light stimulation. It follows that such stimulation is not required for the formation of the basic structure of the rod, although it may play a role in the functional maturation of the rod-sacs.

The clavate projections found at the basal body are similar to structures reported in the centriole of the leucocyte (8, 9).

The nine delicate short stalks connecting the distal periphery of the basal body with the adjacent plasma membrane seem to affix the basal body, and maintain it in a permanent perpendicular orientation to the plasma membrane. It is noteworthy that at the beginning of the morphogenetic process only the centriole that will become the basal body of the primitive cilium develops these connections with the cell membrane.

The dense bands in the plasma membrane of the connecting cilium and the connections between these bands and the ciliary tubules might keep the corresponding structures at a nearly constant distance and thus maintain the tubules approximately parallel to one another. Precise relationship between ciliary tubules and ciliary membrane might be important for an orderly morphogenetic process as indicated by preliminary observations on the formation of abnormal retinal rods (10). The function of the cross-striated bundle of fine filaments observed beneath the basal body is unknown at present.

Although the present description is primarily concerned with the outer segment of the retinal rod of the kitten, a study of rod morphogenesis in mouse retina reveals essentially the same process. Rod morphogenesis in other mammals probably follows the same pattern, but certain modifications may be expected in lower vertebrates since it is known that the structure of their mature rod-sacs is different (3).

The authors would like to express their appreciation to Dr. G. E. Palade for critical reading of the manuscript and also for his valuable suggestions for its revision.

Bibliography

1. Sjöstrand, F. S., *J. Cell. and Comp. Physiol.*, 1953, **42,** 15.
2. De Robertis, E., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 319.
3. Yamada, E., *Kurume Med. J.*, 1957, **4,** 127.
4. Yamada, E., Tokuyasu, K., and Iwaki, S., *J. Kurume Med. Assoc.*, 1958, **21,** 1979.
5. De Robertis, E., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** No. 4, suppl., 209.
6. Caulfield, J. B., *J. Biophysic. and Biochem. Cytol.*, 1957, **3,** 827.
7. Sjöstrand, F. S., *J. Cell. and Comp. Physiol.*, 1953, **42,** 45.
8. Yamada, E., *Proc. 1st Regional Conf. Electron Micr. in Asia and Oceania*, Tokyo, 1956, 247.
9. Bessis, M., Breton-Gorius, J., and Thiéry, J. P., *Rev. Hématol.*, 1958, **13,** 363.
10. Tokuyasu, K., and Yamada, E., in preparation.
11. Fawcett, D. W., and Porter, K. R., *J. Morphol.*, 1954, **94,** 221.

Explanation of Plates

All figures are electron micrographs of retinal rods of kittens from 2nd to 4th day of age before the opening of the eyes, except Figs. 21 and 22 which show retinal rods of the adult rabbit.

Plate 121

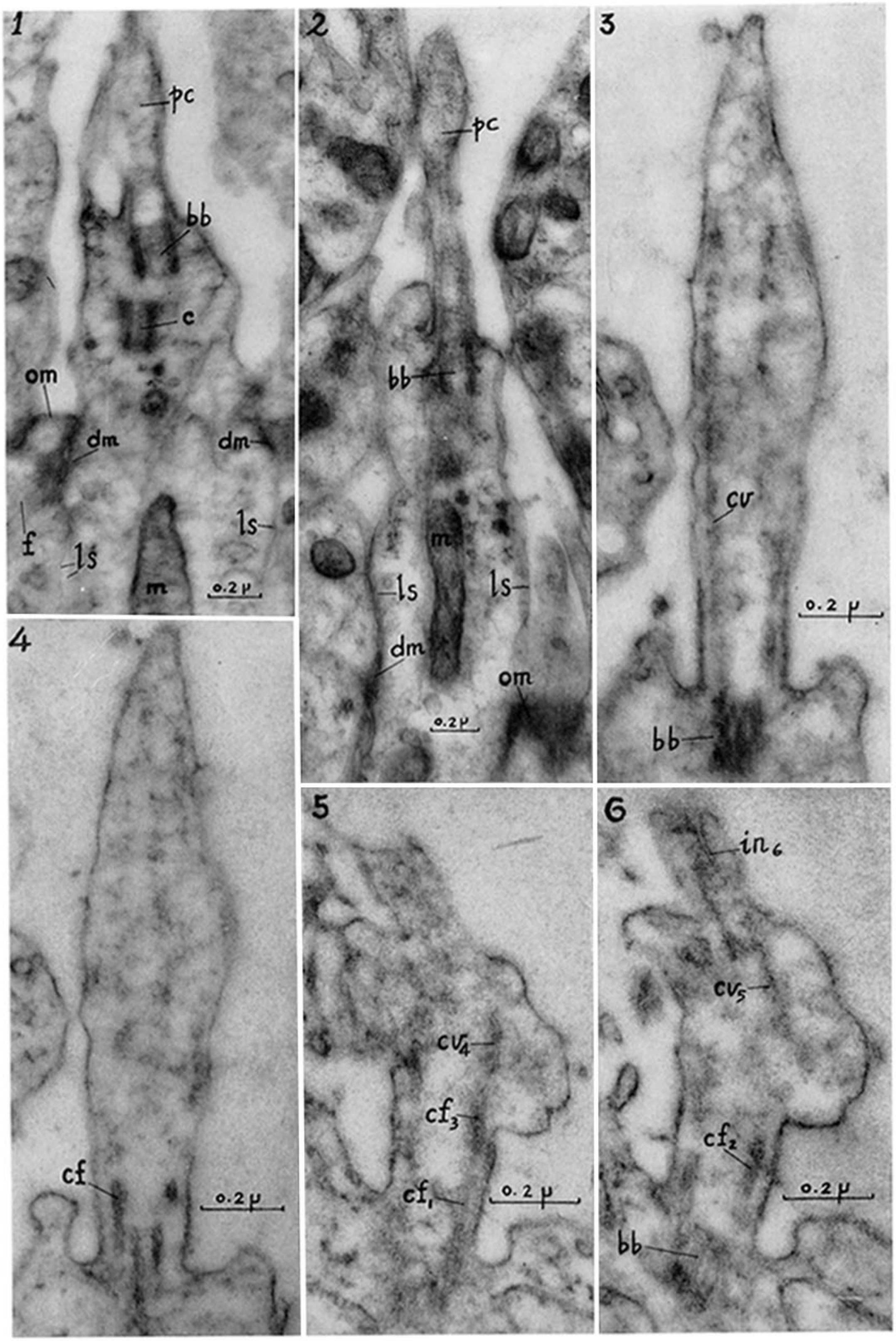
Fig. 1. The distal portion of the visual cell protrudes through the outer limiting membrane (*om*) and two centrioles appear in the distal portion. One of them, closer to the distal end of the protrusion than the other centriole (*c*), extends ciliary tubules, thus forming the primitive cilium (*pc*) and becomes the basal body (*bb*) of the cilium. Mitochondria (*m*) are still beneath the outer limiting membrane at this stage. An accumulation of a dense material (*dm*) equivalent to that found in epithelial terminal bars is observed at the level of the outer limiting membrane under the lateral surfaces of neighboring cells (*ds*) and projects fine filaments (*f*) into the cytoplasm. × 40,000.

Fig. 2. The primitive cilium (*pc*) and also the protrusion of the visual cell extended further distally than those in Fig. 1. The distal region of the primitive cilium tends to enlarge and the cilium shows a somewhat scoop-like profile. A mitochondrion (*m*) is now above the outer limiting membrane (*om*). For other terms, see the explanation of Fig. 1. × 37,000.

Figs. 3 and 4. Serial sections. Note that a ciliary tubule (*cf.* in Fig. 4) extends from the basal body (*bb* in Fig. 3) for about 0.3 μ into the primitive cilium, then becomes a row of small vesicles (*cv* in Fig. 3). These vesicles are called ciliary vesicles. × 64,000.

Figs. 5 and 6. Serial sections. The plasma membrane of the distal region appears quite irregular. Note that a ciliary tubule (cf_1 in Fig. 5, cf_2 in Fig. 6, and cf_3 in Fig. 5), ciliary vesicles (cv_4 in Fig. 5 and cv_5 in Fig. 6), and an infolding of the plasma membrane (in_6 in Fig. 6) are disposed in a single row. *bb;* basal body of the primitive cilium. × 70,000.

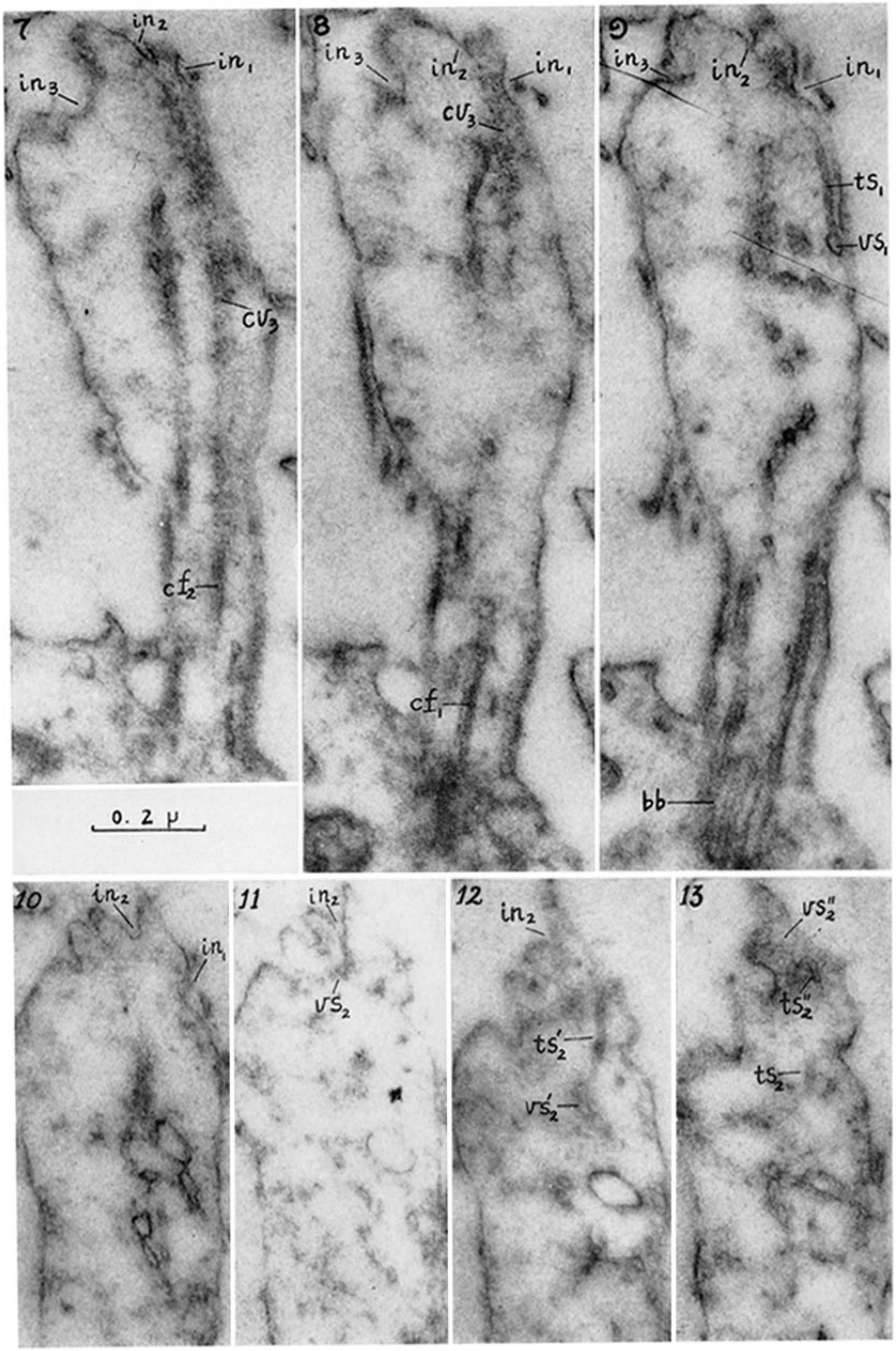
THE JOURNAL OF
BIOPHYSICAL AND BIOCHEMICAL
CYTOLOGY



(Tokuyasu and Yamada: Fine structure of retina. IV)

PLATE 122

FIGS. 7 to 13. Serial sections. Note that a ciliary tubule (cf_1 in Fig. 8 and cf_2 in Fig. 7), ciliary vesicles (cv_3 in Figs. 7 or 8), and infoldings of the plasma membrane (in_2 in Figs. 7 and 8) are disposed on the single row. A similar relationship is recognized for in_3 in Figs. 7 to 9. Notice also that membranous structures of tubular or vesicular nature are observed forming rows with the bottoms of the infoldings, *e.g.*, vs_1, ts_1, and in_1 in Fig. 9, or vs_2' in Fig. 12, ts_2 in Fig. 13, ts_2' in Fig. 12, ts_2'' in Fig. 13, vs_2'' in Fig. 13, and in_2 in Fig. 12, or in continuity with the bottoms (vs_2 and in_2 in Fig. 11). *bb;* basal body of the cilium. × 88,000.



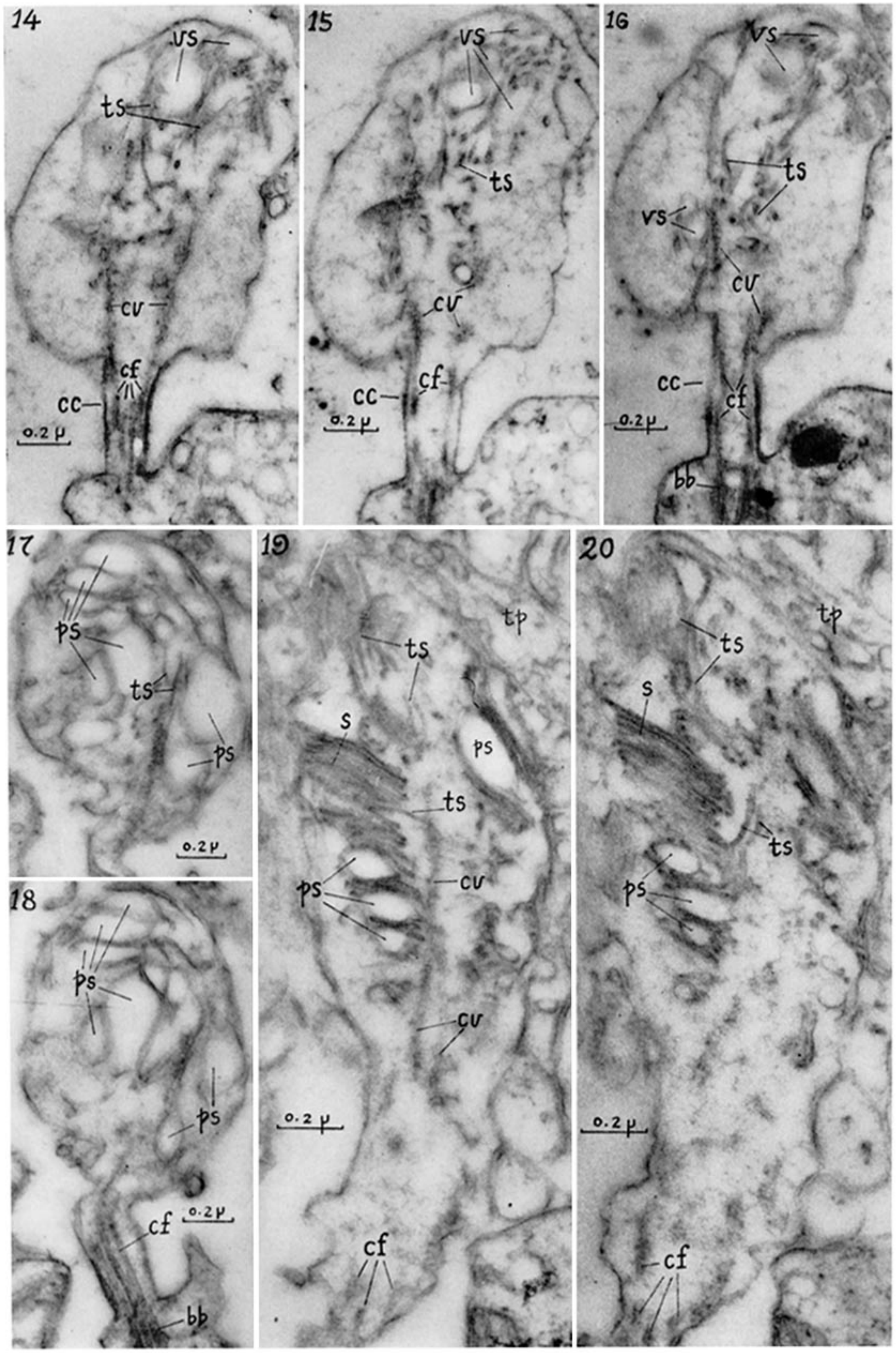
(Tokuyasu and Yamada: Fine structure of retina. IV)

Plate 123

FIGS. 14 to 16. The distal region of the primitive cilium widens to about 1 μ and shows the primitive shape of the outer segment of the rod. Its basal portion does not change its original form and becomes the so called connecting cilium (*cc*) of about 0.5 μ in length. Numerous tubular structures (*ts*) and a small number of vesicular structures (*vs*) are disposed in such a way that they appear to be directed towards the tip of the outer segment. The plasma membrane covering the distal region of the outer segment becomes smooth again at this stage. Note that the ciliary vesicles (*cv*) appear to fade in the distal region and do not seem to reach the distal plasma membrane. $\times$ 41,000.

FIGS. 17 and 18. Serial sections. Tubular structures (*ts*) are a few at this stage and instead, large vesicular structures become predominant in the outer segment. These structures seem to be the so called "primitive rod sacs" (*ps*). They show somewhat extended oval or spindle-like profiles often with sharply pointed ends.

FIGS. 19 and 20. Serial sections. At this stage, such primitive rod sacs as shown in Figs. 17 and 18 seem to flatten taking the shape similar to that of mature rod sacs. They (*s*) are arranged into the transverse position to the long axis of the outer segment and some of them appear to be continuous with tubular structures (*ts*). Several primitive rod sacs (*ps*) remain unflattened. Notice that the mass of the flattened rod sacs occupies the region approximately corresponding to that of the mass of the primitive rod sacs in Figs. 17 and 18, or to that of the tubular structures in Figs. 14 to 16. Numerous tubular structures are found in the distal region of the outer segment above the mass of the flattened rod sacs. The ciliary vesicles (*cv*) appear to fade in the central region and do not seem to extend into the distal region of the outer segment. *cf;* ciliary filament, and *tp;* tortuous processes of the cell membrane of the pigment epithelium surrounding the outer segment. $\times$ 51,000.



(Tokuyasu and Yamada: Fine structure of retina. IV)

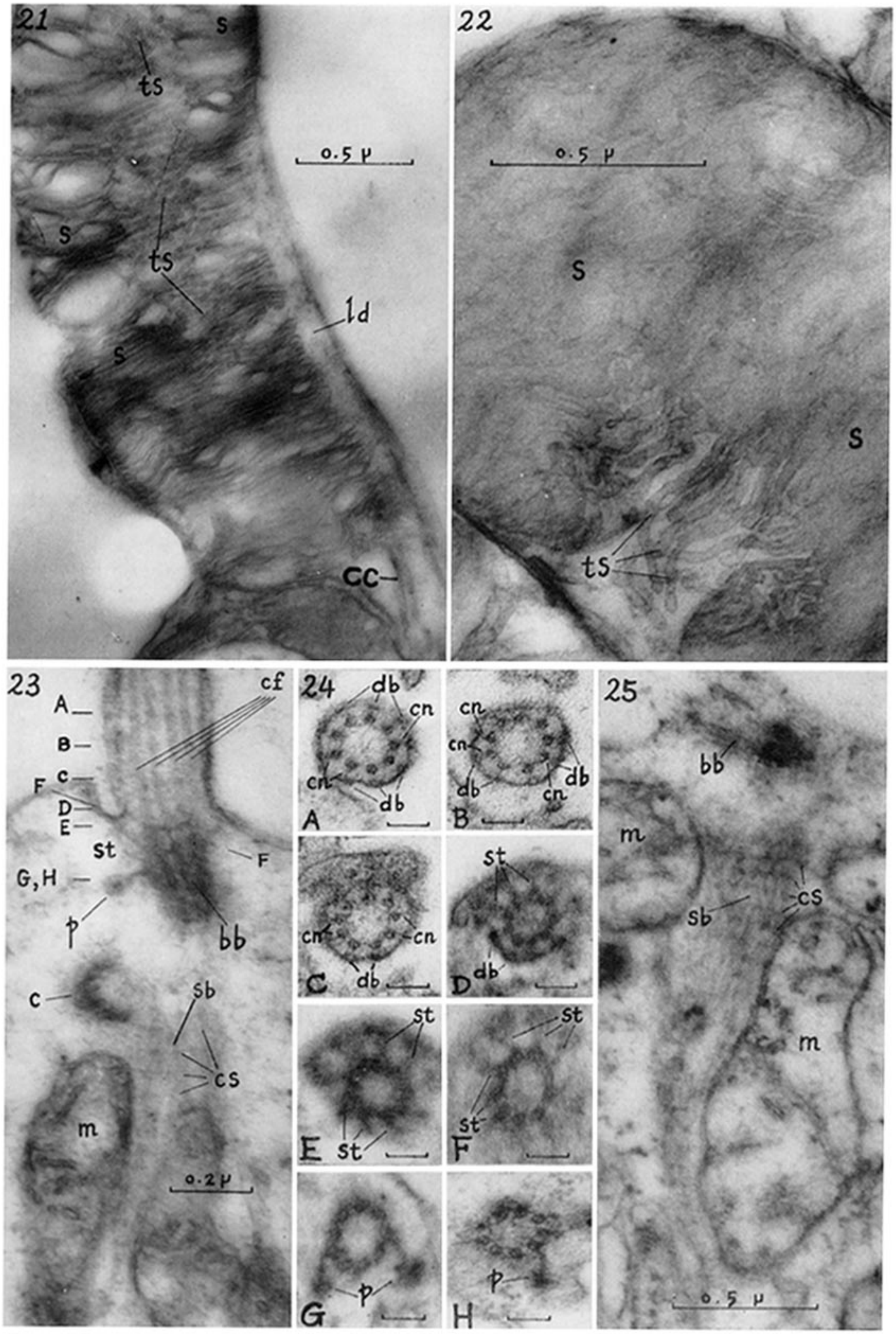
Plate 124

Figs. 21 and 22. Longitudinal and oblique sections of outer segments of retinal rods of the adult rabbit. Tubular structures (*ts*) remain numerous in the mature outer segments and are obviously continuous with the rod sacs (*s*). Note that in Fig. 21, the space of low density (*ld*) where the ciliary filaments or vesicles are supposed to be disposed extends only a few micra into the outer segment. *cc;* connecting cilium. Fig. 21, × 37,000; and Fig. 22, × 68,000.

Fig. 23. Longitudinal profile of the basal body and connecting cilium. Fine filamentous short stalks (*st*) connect the upper end of the basal body with the adjacent plasma membrane and a clavate projection (*p*) extends out of the trunk of the basal body (*bb*). The short stalks have a length of about 60 to 100 mμ and a width of about 10 to 15 mμ. The diameter of the "clubbed" part of the clavate projection is about 60 to 80 mμ, and the "handle" part measures about 60 to 100 mμ in length and about 15 to 20 mμ in width. The end of the other centriole (*c*) is cut obliquely in this section. A bundle of fine filaments (*sb*) is observed beneath the basal body and it shows slight cross-striations (*cs*) with a periodicity of about 55 mμ. *cf;* ciliary tubules. × 65,000.

Fig. 24. Cross-sections of the basal body and connecting cilium at various levels, selected from many series of serial sections. Figs. *A* to *C* are serial sections. Figs. *A* to *H* seem to correspond each to the cross-sections at levels indicated at *A* to *H* in Fig. 23. The structures *st* and *p* defined in the explanation of Fig. 23 are observed correspondingly in each section of Fig. 24. Note that the number of *st* is nine (Fig. *E*) and also that each of them seems to extend from the position where each ciliary tubule is projected from the basal body (Figs. *D* and *F*). The plasma membrane of the connecting cilium appears to be denser along each ciliary tubule than in between (Figs. *A* to *C*). Between each ciliary tubule and the corresponding dense band (*db*), a connection (*cn*) is sometimes observed (Figs. *A* to *C*). The scale indicates 0.1 μ. Each, × 65,000.

Fig. 25. A cross-striated bundle of the fine filaments (*sb*) is found beneath the basal body (*bb*). The periodicity of the cross-striation (*cs*) is about 55 mμ. *m*, mitochondria. × 48,000.



(Tokuyasu and Yamada: Fine structure of retina. IV)