FILAMENTOUS STRUCTURES IN THE INNER SEGMENT OF HUMAN RETINAL RODS

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Filamentous structures have been described repeatedly in the inner segment of vertebrate retinal rods (Sjöstrand, 1953; Tokuyasu and Yamada, 1959; Missotten, 1960; Cohen, 1960, 1961). In most cases, one or two fibers about 500 A in diameter and with a 650 to 700 A repeating period have been shown; the characteristics of the period are slightly variable in the descriptions of the above authors. The fibers seem to originate from the centrioles and deepen into the inner segment towards the outer limiting membrane of the retina; their ending has not yet been clearly established.

Similar to the above structures are the root and rootlets in the dendrite of the insect acoustic neuron (Gray and Pumphrey, 1958) and the fibrous ciliary rootlets of invertebrate animals (Fawcett, 1958).

In the course of extensive electron microscope investigations on the normal human retina, some new data on the fine structure of the inner segment filaments have been acquired, which are reported in this note.

MATERIALS AND METHODS

Normal extramacular retina from two partially light-adapted human eyes was used in the research. The eyes were enucleated from two male patients, aged 54 and 57 years, suffering from choroidal melanoma and malignant orbital neoplasia, respectively. The preparation of the eyes, including embedding in epoxy resin (Araldite), was the same as that reported in a previous paper (Bairati and Orzalesi, 1963). Fixation was always started within 5 minutes of enucleation of the eyes.

Longitudinal and tangential ultrathin sections (300 to 600 A thick) were cut on an LKB (Ultrotome) microtome with glass knives. The sections were stained with 2 per cent uranyl acetate in 50 per cent ethanol, or with 1 and 5 per cent phosphotungstic acid in 50 per cent ethanol, or with potassium permanganate according to the method of Lawn (1960). They were examined and photographed with a Siemens Elmiskop II at primary enlargements ranging from 1500 to 30,000. Further photographic enlargement was effected when needed.

RESULTS AND DISCUSSION

Longitudinal sections of human retinal rods show filamentous structures arranged in bundles running parallel to the major axis of the photoreceptor in the inner segment (Fig. 1). Similar structures have not been observed in any of the longitudinal sections of cones examined during this work.

The bundles of filaments originate from the centrioles placed at the outer end of the inner segment (Fig. 2). One of the centrioles located peripherally supports the connecting cilium structure, while the other appears, in most cases, lateral to it and is frequently oriented perpendicularly with respect to the former; with less frequency, however, the second centriole can show a different situation and orientation. Vesicular elements of smooth-surfaced endoplasmic reticulum are found surrounding the centrioles (Fig. 2). The filaments do not seem to start directly from the centriole fibers, but rather from the sides and lower end of the granular electron-opaque material wrapping the centrioles (Fig. 2).

After their point of origin the two bundles of filaments remain distinct only in the initial tract, joining then, in most cases, in a single large bundle running a rectilinear course along the ellipsoid. In this zone the filaments are in close relationship with the numerous elongated mitochondria of the inner segment (Figs. 1 and 7). Cross-sections of the ellipsoid show clearly that the filaments of the bundle are not regularly packed in a compact fiber, but merely adapt themselves to the narrcw cytoplasmic spaces among the mitochondria (Fig. 5). In most cases the bundle is situated at the center of the ellipsoid but it can also be observed peripherally. About 200 filaments are found forming the bundle at the level of the ellipsoid.

When passing from the mitochondrial zone to the myoid, the bundle of filaments becomes thinner and is observed with less frequency in longitudinal sections. About 50 filaments constitute the bundle at this level. In the myoid the bundle appears surrounded by numerous vesicles of the endoplasmic reticulum and of the Golgi apparatus (Fig. 3), but direct connections between the filaments and these structures (Cohen, 1960, 1961) are not observed. On the basis of the present observations it seems possible that the bundle becomes progressively reduced in size as it traverses the inner segment, until it ends freely in the cytoplasm of the myoid at the level of the Golgi zone.

The cross-striated appearance of the bundles of filaments is caused by the periodic alternation of clear and dark zones (Figs. 1, 4, and 6). The 700 A period comprises a 250 A clear zone and a 450 A dark zone (Figs. 4 and 6). The characteristics of the period, like those of the filaments, are fairly constant along the whole bundle (Figs. 1 and 3). The filaments in the bundle are parallel and continuous, this being particularly evident where few filaments are seen running isolated between two mitochondria (Fig. 7). Each filament shows a rounded section 40 to 60 A in diameter, adjacent filaments being separated by a 100 to 150 A space (Fig. 5). In cross-sections of the bundles, neither a particular symmetry in the disposition of the filaments nor any peculiar connecting structures among the filaments are clearly observed (Fig. 5). In longitudinal sections each filament shows zones of variable thickness, being thicker at the level of the dark zones of the period and thinner in the clear zones (Fig. 6); the zones of different thickness of each filament correspond exactly with those of the adjacent ones (Fig. 6). Moreover, the spaces among the filaments in the bundle exhibit low electron opacity in the clear zones of the period, while in the dark zones they appear filled with an electron-opaque material (Figs. 4 and 6). Staining with phosphotungstic acid (PTA) increases slightly the contrast of the filaments, mostly in the dark zones; besides, PTA-stained filaments show more evident globular substructure than do filaments stained with uranyl acetate. On the contrary, staining with potassium permanganate results in poor contrast of the filamentous structures.

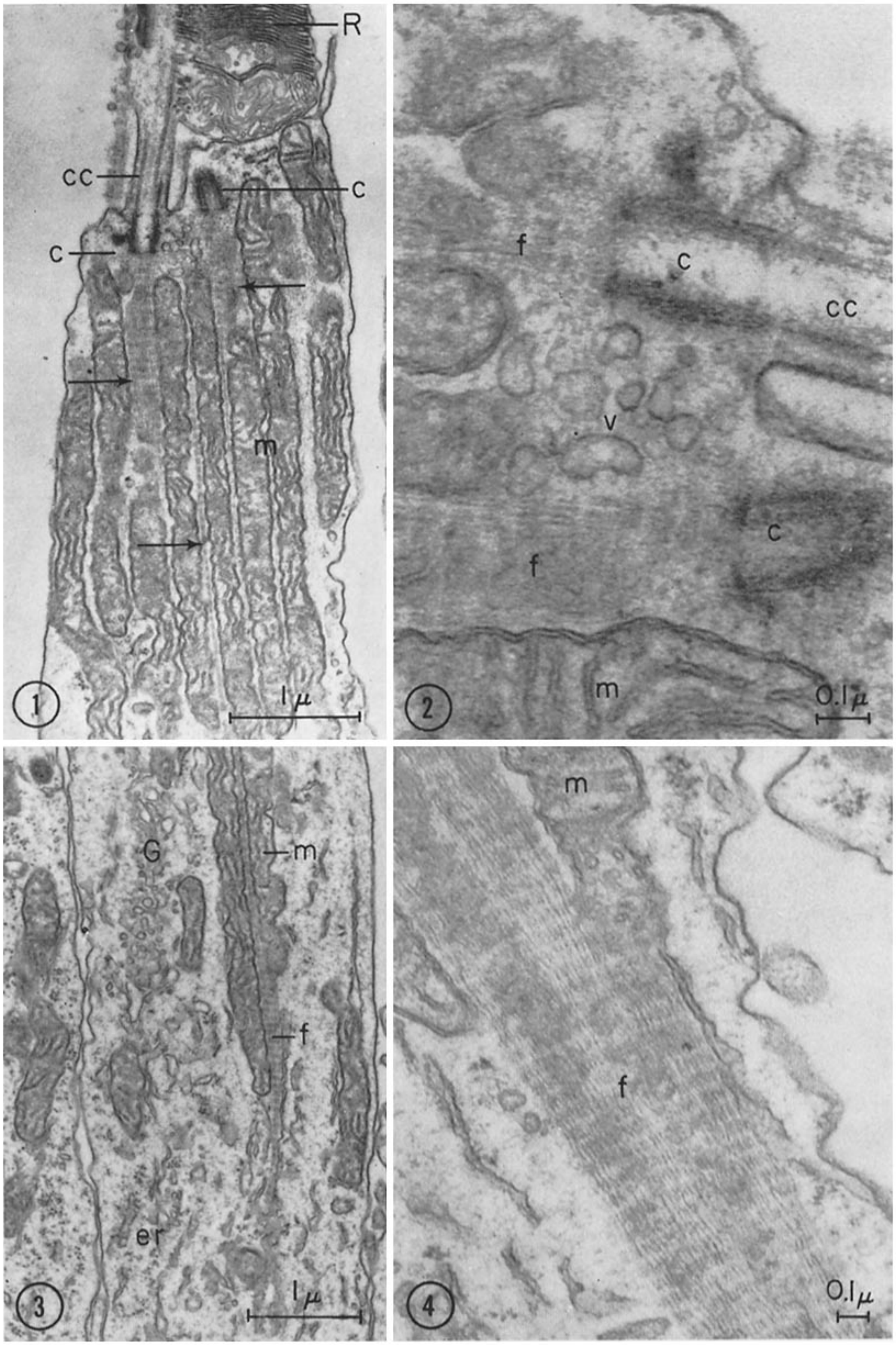
Based on the above morphological observations, several conclusions are possible: (*a*) The inner segment of human photoreceptors contains bundles of filaments, with a peculiar periodic structure, that are probably present only in rods. The periodic aspect of the bundles depends on the characteristics of both the component filaments and the spaces among them. It is most probable that these bundles of filaments correspond to the fibers with periodic structure reported by previous authors (Sjöstrand, 1953; Missotten, 1960), in

FIGURE 1 Longitudinal section through the ellipsoid of a human retinal rod showing striated bundles of filaments (arrows) running among the mitochondria (*m*) of the inner segment. *R*, rod outer segment; *c*, centriole; *cc*, connecting cilium. × 20,000.

FIGURE 2 Upper portion of a rod inner segment at higher magnification. Note the structure of the centrioles (*c*) and the origin of the bundles of filaments (*f*). *cc*, connecting cilium; *v*, vesicles of endoplasmic reticulum; *m*, mitochondrion. × 80,000.

FIGURE 3 Longitudinal section through a rod myoid showing the ending of the bundle of filaments (*f*). *m*, mitochondria; *G*, Golgi zone; *er*, endoplasmic reticulum. × 17,000.

FIGURE 4 Longitudinal section through a wide bundle of filaments (*f*) in a rod ellipsoid, showing the periodic alternation of clear and dark zones along the bundle. *m*, mitochondrion. × 45,000.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the inner segment of mammalian and human rods. The evidence of a peculiar filamentous substructure of these fibers is probably related to improvements in fixation and embedding technique. It is also possible that most of the similar fibrous structures, which have been reported in association with centrioles of cilia and flagella (Fawcett, 1958) and in the acoustic neuron of insects (Gray and Pumphrey, 1958), would show an analogous elementary ultrastructure if more suitable techniques of preparation were used. (*b*) The filaments of the bundles, with respect to their structure and periodicity, do not bear any similarity to collagen fibrils or epithelial tonofilaments. On the other hand, these filaments show remarkable analogy with the striated myofilaments, supporting the hypothesis that they are made up of contractile proteins. A similar suggestion was advanced also for the ciliary rootlets of the endostilar cells of lower chordates based on the ultrastructural features of these rootlets and their relationships with mitochondria (Olsson, 1962). Recently, contractile proteins (like actin and myosin) have been suggested as components of the filamentous structures of flagella of human spermatozoa (André and Thiéry, 1963); however, using antimyosin and anti-actin fluorescent antibodies, actin and myosin were not detectable in sperm tails and tracheal cilia (Finck and Holtzer, 1961). (*c*) Fibers with cross-banded structure associated with centrioles of cilia and flagella (ciliary roots and rootlets) and of visual and acoustic receptors have mostly been considered as statical apparatus for anchoring the centriole itself (Fawcett, 1958). Beyond this function, in the case of receptors, the possibility of conduction of intracellular stimuli along these structures has been advanced (Sjöstrand, 1953; Cohen, 1960). It can now be suggested that all these structures, if made up of contractile proteins, could exert, beyond a supportive function, also dynamic action. In the case of photoreceptors this could be related to the movements of the outer and inner segments associated with changes in the adaptation state of the retina (Detweiler, 1943). The complexity of the argument and the scarce physiologic information available on the subject render further research necessary before a satisfactory interpretation of these structures can be reached.

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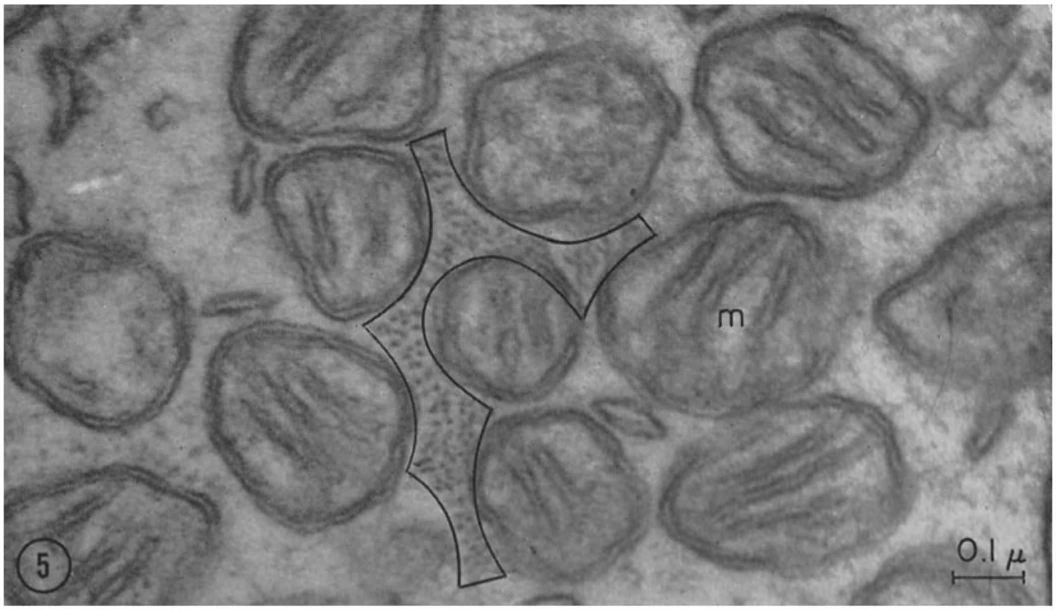
Figure 5 Cross-section of a rod ellipsoid showing the cross-sectioned bundle of filaments (outlined) and its relationship with the mitochondria (*m*). × 95,000.

Figure 6 Higher magnification of the bundle of filaments in longitudinal section. Note the characteristics of the filaments and of the matrix among them in the periodic dark (*d*) and clear (*c*) zones of the bundle. × 225,000.

Figure 7 Higher magnification of a few filaments (arrows) running isolated among the mitochondria (*m*) of the ellipsoid. × 165,000.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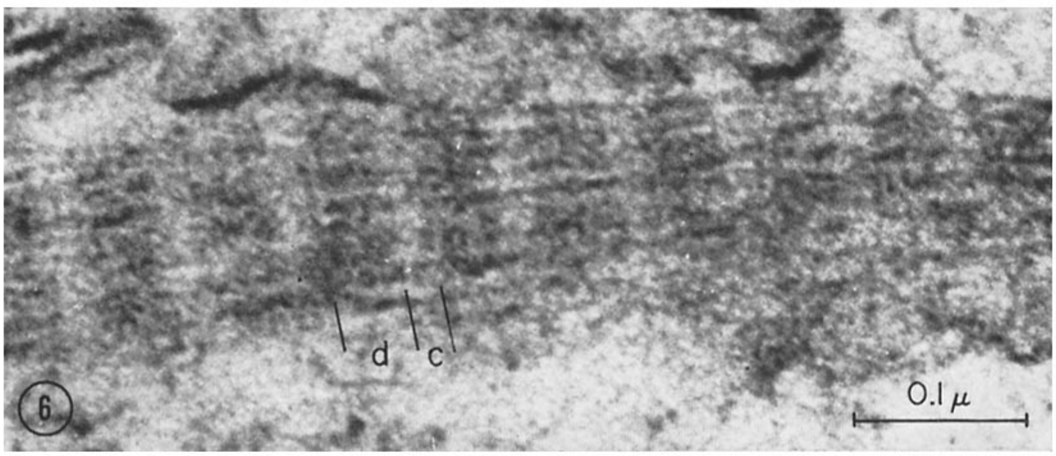
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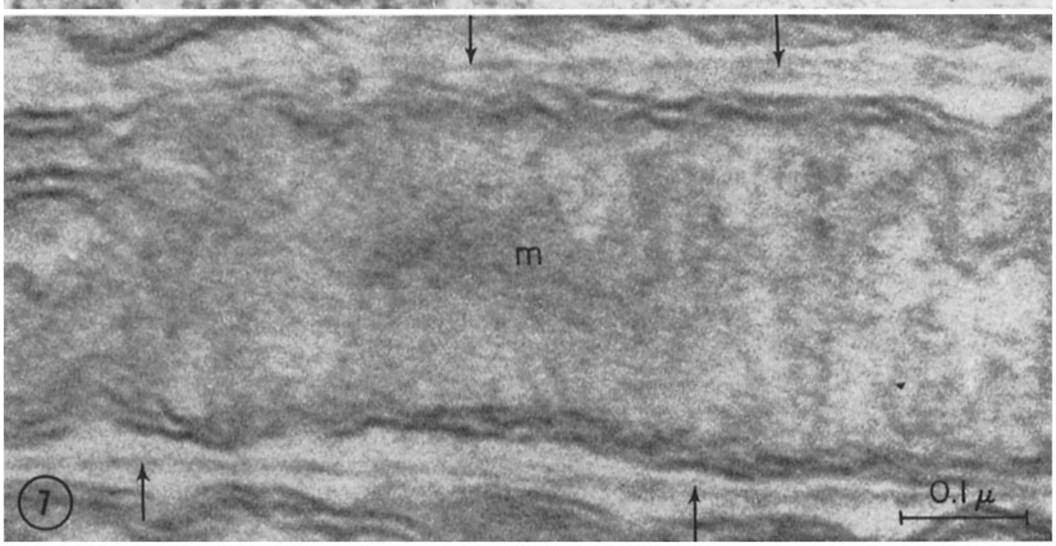
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